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TIME-TEMPERATURE RELATIONS IN DIFFERENT TYPES OF PEACH-ROT INFECTION[1]

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INTRODUCTION

Car-lot shipments of peaches frequently show considerable spoilage upon arrival at their destination, and this spoilage is usually in the form of Monilia and Rhizopus rots. The behavior of these rots at various constant temperatures was described in an earlier publication.[2] Data were also reported on the effect of delays in cooling. The present paper reports further studies on the temperature responses of Monilia and Rhizopus, includes experimental data on their behavior in a gradually falling temperature such as prevails in a refrigerator car, shows the contrasts in the incubation period at different temperatures and with different types of inoculation, and attempts to equate the different growth and incubation values. It also gives shipping results from sprayed and unsprayed fruit.

EQUATION OF TEMPERATURE VALUES

METHODS

Experiments were made at various constant temperatures with different types of inoculation, and the various responses were reduced to a basis of comparison. Three different methods of inoculation were used: (1) Forcing the spores into the peaches with a needle, (2) puncturing the peaches and then dusting them with spores, and (3) dusting unpunctured peaches.

The needle inoculations were made by pushing spores to a depth of 3 to 6 mm. into the flesh of the peach with a coarse needle. The dusting inoculations were made by placing the fruit in a large paper bag along with rotten but firm peaches carrying a good covering of spores, and rolling the two lots gently back and forth from one end of the bag to the other. In the Rhizopus dusting experiments, fruiting Petri-plate cultures were sometimes substituted for the rotten peaches as inoculation material.

As mentioned above, a part of the dusted peaches had been previously punctured. Except where otherwise stated, 10 punctures were made on each peach with No. 18 wire (paper-clip wire). As a matter of convenience wires were mounted in a large cork at distances of 12 to 15 mm. apart, and this group of wires was forced first into one side of the peach and then into the other.

[1] Received for publication Aug. 17, 1928; issued December, 1928.
[2] BROOKS, C., and COOLEY, J. S. TEMPERATURE RELATIONS OF STONE FRUIT FUNGI. Jour. Agr. Research 22: 451-465, illus. 1921.

The peaches used in the experiments were selected with great care so that those of the different lots would be alike in maturity, in size and color, and in freedom from evident bruises and blemishes. Eight to ten peaches were used under each condition tested, and most of the experiments were repeated fifteen to twenty times. Before the inoculations were made [3] the peaches were cooled to approximately the temperature at which they were later to be placed.

The fungi used in the inoculations were obtained from active peach rots. They were apparently *Sclerotinia fructicola* (Wint.) Rehm and *Rhizopus nigricans* Ehr. and are referred to in the present paper as Monilia and Rhizopus.

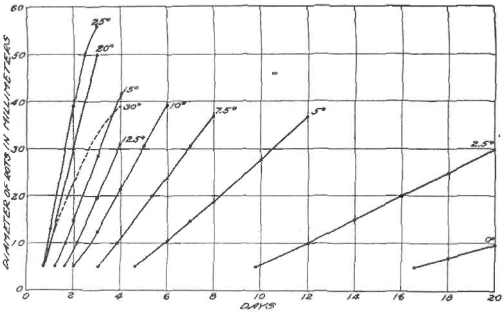

FIG. 1.—Growth curves of Monilia needle-inoculation rots on peaches at various constant temperatures; the average of results from 20 series of experiments, 1 with Carman, 4 with Hiley, 8 with Belle, and 7 with Elberta. *There was little contrast between the different varieties, but* the results from the individual experiments sometimes varied as much as 2 per cent from the average in the rate of growth and 10 per cent from the average in the period of incubation. In the growth at 30° C. and in the incubation period at 0° and 2.5°, the variation was sometimes even greater than this. The results reported are based on peaches showing 10 to 16 pounds pressure with a standard pressure tester, but similar results were obtained at the higher temperatures and only slightly accelerated development at the lower temperatures with peaches showing 5 to 10 pounds pressure

MONILIA NEEDLE INOCULATIONS

The results with the Monilia needle inoculations at constant temperatures are shown in Figure 1. It will be seen that considerable time was required for the rots to become established, especially at the lower temperatures, but that after this incubation period they increased in diameter in a fairly uniform manner, requiring approximately the same number of hours to enlarge from a 10 mm. to a 20 mm. diameter as to enlarge from 20 mm. to 30 mm. Usually the same rate of growth continued upward to a diameter of 40 mm. or more and downward to a diameter of 5 mm. At 30° C., however, there was a continual slowing up of the growth rate after the rot had attained a diameter of 15 mm. At some of the lower temperatures the rate of increase in diameter was not always as rapid between 5 mm.

[3] In an earlier report the inoculations were made on warm peaches. BROOKS, C., and COOLEY, J. S. Op. cit.

and 10 mm. as in the later stages of growth, but this difference has largely disappeared in the averages, as shown in Figure 1.

The rate of increase in diameter at the different temperatures is graphically shown in the smoothed curve (*a*) of Figure 2. This curve is based on the growth values shown in Figure 1 and has been found to hold both for rots started at the given temperatures and for those started under room or field conditions and then transferred to these temperatures.

It is obvious that with a uniform increase in diameter at a particular temperature, as described above, there was a progressive increase in the volume of work accomplished per hour as the rot enlarged, the actual quantity of tissue broken down in one hour during the 20 to 30 mm. stage being much greater than that broken down in one hour during the 10 to 20 mm. stage. The rots at the higher temperatures, therefore, are soon in a later and a more enlarged period of growth than those at the lower temperatures, making any temperature comparison invalid if based on volume of rot or weight of rotten tissue or on actual work accomplished in a specified hour, at a considerable period after inoculation. Comparisons based on the rate of increase in diameter, however, are not subject to this criticism, since, as pointed out above, the rate of increase is usually practically the same over the larger part of the period of growth.

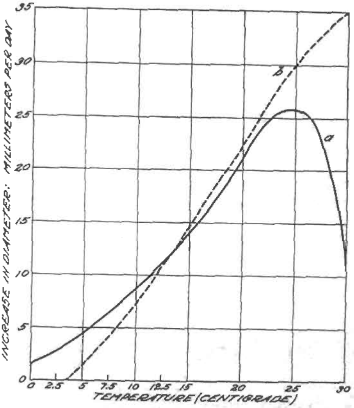

FIG. 2.—Temperature curves based on the hourly increase in the diameter of the rots as shown in Figures 1 and 9. Curve *a*, Monilia; *b*, Rhizopus

Probably the best basis for any temperature comparison is that of the number of hours required to complete a particular stage of development, especially in any study involving the incubation period. It is interesting to note that at temperatures from 15° to 25° C. approximately the same number of hours were required for the Monilia rots to get started and grow to a diameter of 10 mm. as for them to enlarge from a diameter of 10 mm. to one of 30 mm.; whereas at the lower temperatures more time was required for the first of these periods of development than for the second and at 30° much more time for the second than for the first. (See Table 1 and fig. 1.)

TABLE 1.—*Rate of development of Monilia rots at different stages and temperatures, based on the growth curves of Figure 1*

Growth period	Approximate number of hours required at stated temperatures (° C.)									
	30°	25°	20°	15°	12.5°	10°	7.5°	5°	2.5°	0°
From time of inoculation up to a diameter of 10 mm	23	21	25	39	51	65	91	142	288	480
From a diameter of 10 mm. to one of 30 mm	39	19	24	36	44	54	73	110	195	------

In developing any temperature equation for Monilia it is evident that the incubation period should be separated from the growth period and that the line of separation should be made at the earliest possible stage. The curves of Figure 1 would seem to justify making this dividing line at the point where the rots have attained a diameter of 5 mm. (0.2 inch). Taking this as the dividing line, it is seen that at 30° C. the incubation period was about 19 hours; at 25°, 17.5 hours; at 20°, 19.5 hours; at 15°, 31 hours; at 12.5°, 40 hours; at 10°, 50 hours; at 7.5°, 75 hours; at 5°, 112 hours; at 2.5°, 235 hours; and at 0°, 400 hours. These values are shown in curve *a* of Figure 3.

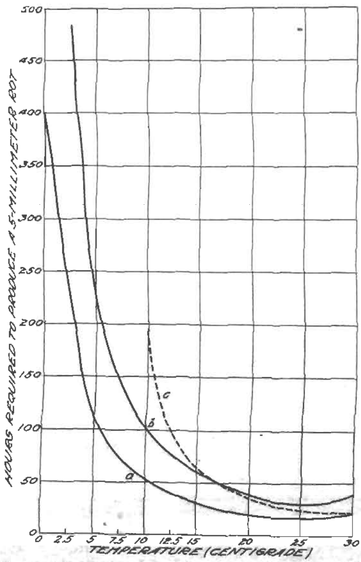

FIG. 3.—Temperature curves based on the average number of hours required to produce a 5-mm. rot as shown in Figures 1, 5, and 7. Curve *a*, Monilia needle inoculations; *b*, Monilia dusting inoculations on punctured peaches; *c*, Rhizopus needle inoculations. Curve *b* can also be regarded as showing the approximate number of hours required to bring the percentage of infection up to 20

Since these records in hours are a measure of the time required to do a certain volume of work, the reciprocals of these hour values give the relative work accomplished at the different temperatures in a given time. These reciprocal values are the basis for curve *a* of Figure 4. It will be seen from this curve that at 5° C. about four times as much work was accomplished as at 0°, about three times

as much at 7.5° as at 2.5°, about 120 per cent more at 10° than at 5°, about 90 per cent more at 12.5° than at 7.5°, about 60 per cent more at 15° than at 10°, about 60 per cent more at 20° than at 15°, about 12 per cent more at 25° than at 20°, and about 10 per cent less at 30° than at 25°.

The above temperature contrasts for the incubation period should be compared with the temperature contrasts for the growth period as shown in curve *a*, Figure 2. A study of this curve shows that the growth at 5° C. was about three times as fast as at 0°, the growth at 7.5° about two and six-tenths times that at 2.5°, the growth at 10° about twice that at 5°, the growth at 12.5° about 75 per cent greater than that at 7.5°, the growth at 15° about 55 per cent greater than at 10°, the growth at 20° about 50 per cent greater than at 15°, the growth at 25° about 25 per cent greater than at 20°, and the growth at 30° less than half that at 25°. It will be seen that at the lower temperatures a 5° change had greater significance in the incubation stage of the fungus than in the growth stage and that with both incubation and growth the effect of a 5° change was far greater at the lower temperatures than at the higher ones.

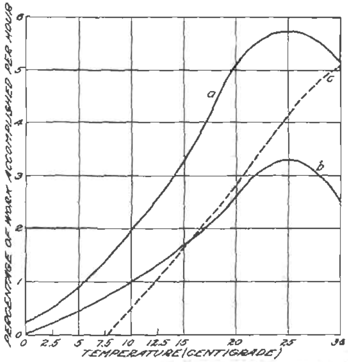

FIG. 4.—Temperature curves for the incubation period, showing the percentage of work accomplished per hour based on the reciprocals of the values shown in Figure 3. Curve *a*, Monilia needle inoculations; *b*, Monilia dusting inoculations; *c*, Rhizopus needle inoculations

MONILIA DUSTING INOCULATIONS

With needle inoculations all of the rots at a particular temperature usually started at practically the same time, but with the peaches that were punctured and dusted some of the rots were often twice as long as others in starting.

The time at which the rots appeared on the punctured and dusted peaches at the various temperatures is shown in Figure 5. At 25° C. about 10 per cent of the punctures had rots by the end of the first day and 76 per cent by the end of the second day; at 20°, 8 per cent the first day, 42 per cent the second day, and 96 per cent the third day; at 30°, 36 per cent the second day and 54 per cent the third day; at 15°, 19 per cent the second day and 78 per cent the fourth day; at 10°, 12 per cent the fourth day and 72 per cent the sixth day; at 5°, about 3 per cent the sixth day, 16 per cent the eighth day, and 29 per cent the tenth day; and at 2.5°, about 6 per cent the thirteenth day and 30 per cent the nineteenth day. It will be seen that the lower

the temperature the greater was the period of time over which infections occurred and that there was a fairly close ratio between the spread of the infection period and the time required to produce the first rot. The final percentage of infection (fig. 5) was much higher at temperatures near the optimum for the fungus than at those distinctly below or above the optimum. This was largely a direct temperature response, but one other factor should be mentioned. The discontinuance of infection records was usually brought about by the absorption of the uninfected punctures in the growth of the rots already started. At the lower temperatures growth was not inhibited to the same relative degree as infection, and therefore the records may have been closed relatively earlier at these temperatures.

The growth curves for the rots resulting from the dusting inoculations on punctured peaches are shown in Figure 6. A comparison of the curves of Figures 5 and 6 with those of Figure 1 shows that the

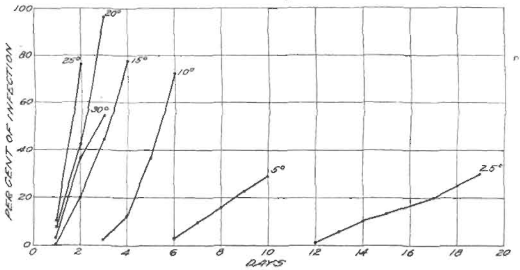

FIG. 5.—Temperature infection curves for peaches dusted with Monilia spores. Each peach had received 10 punctures previous to dusting, and the curves show the percentages of infection at these punctures after the various periods of time indicated on the base line. The results are the average of 16 series of experiments. (See legend of fig. 6)

rots from the dusting inoculations were greatly delayed as compared with those from the needle inoculations. This is brought out more strikingly in the curves of Figure 3, showing the number of hours required for the diameters of the rots to reach an average of 5 mm. and those of Figure 4, showing the relative volume of work accomplished per hour during the incubation period. It will be seen that it took approximately twice as long for a rot to get started when the spores were dusted over a puncture as when they were forced into the flesh with a needle. At 25° C. it did not take quite twice as long, and at 5° and 2.5° it took more than twice as long.

These incubation values for the dusted peaches are based on the number of hours required for the average diameter to reach 5 mm. and are not identical with values obtained by taking an average of the number of hours required to produce a 5-mm. rot. With the latter method of computation all of the rots that eventually appeared at the punctures would be considered in the earlier as well as the later

valuations, and the number of hours required for the incubation period as shown in Figure 3 would be thus increased approximately 30 per cent. This computation would make the incubation period for the punctured and dusted peaches more than two and a half times as long as that for the needle inoculations, instead of twice as long, as reported above.

Two reasons can be assigned for the earlier development of the rots from needle inoculations than from puncturing and dusting: (1) The needle inoculations carried more spores into the flesh of the peach than would be lodged around a single puncture by the dusting method, and (2) a spore located in the moist, broken tissue of the flesh has much more favorable conditions for quick germination and the rapid production of a rot than one located on the surface of the peach at or near a puncture.

A comparison of the curves of Figure 6 with those of Figure 1 would indicate that the growth rate of the rots resulting from dust

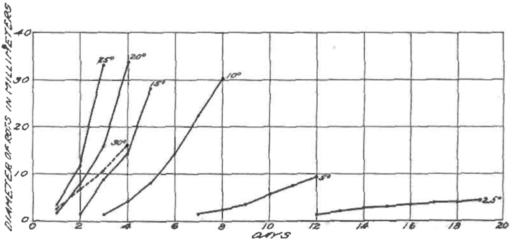

FIG. 6.—Growth curves of rots produced at various constant temperatures by dusting punctured peaches with Monilia spores. The results are the average of 16 series of experiments; 4 with Hiley, 7 with Belle, and 5 with Elberta. There was little contrast between the different varieties, but the results of the individual experiments sometimes varied as much as 50 per cent from the average, and at 5° C. and below the variation was greater than this. These variations, however, were largely between one series of experiments and another with the temperature ratios of the different series remaining fairly uniform. The results as reported are based on peaches showing 10 to 16 pounds pressure, but rots have developed with only slightly more freedom and rapidity on peaches showing 5 to 10 pounds pressure

inoculations was slower than that of the rots from needle inoculations; but such was not the case. The rots when once established grew at practically the same rate, regardless of the method of inoculation; and the relatively slow growth rate indicated for the early stages of the dust inoculations is due to the fact that the continual appearance of new rots prevented the increase in the average diameter from being a true record of the actual rate of growth.

As previously mentioned, apparently sound peaches as well as punctured ones were included in the dusting experiments. A record was also kept of the rots developing on the punctured peaches at points other than the punctures and of those developing on check peaches, which were neither punctured nor dusted. The results of these experiments have been extremely variable and very difficult to interpret. In most cases practically no rots developed from dusting unpunctured peaches with Monilia spores, but in some instances there were one-fourth to one-half as many rots on the unpunctured

peaches as on the punctured ones. These rots were usually consid erably delayed as compared with those occurring at the pucturės.

It is possible that the extreme variation in these dusting experiment was due in part to a difference in the condition of the rotten peache used as inoculation material, as it was observed that any juice o small particles of flesh from the rotten peaches might be an aid t infection when lodged upon the sound ones. Great care was taken however, to avoid this by selecting inoculating material that was in firm condition. It is also possible that rolling the peaches back an forth in the bags sometimes resulted in small punctures or abrasion that were of materia assistance in the start ing of the rots. Th writers do not questior the ability of germin ating Monilia spores t gain entrance to th peach without the ai of punctures, but th variable and ofte negative data tha they have obtained o apparently soun peaches, as compare with the almost cer tain infection in th presence of punctures convince them tha Monilia spores are no as great a source of in fection during th usual periods in tran sit as is commonly supposed, except when aided by bruises, punc tures, or similar favoring conditions.

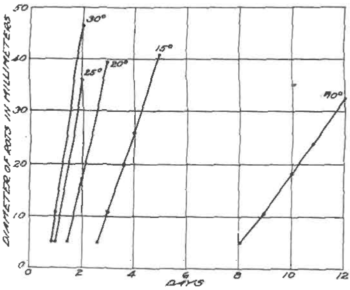

FIG. 7.—Growth curves of Rhizopus needle-inoculation rots; based on the results of 12 series of experiments, 2 with Carman, 1 with Hiley, 5 with Belle, and 3 with Elberta. (For variations see figs. 8 and 9)

RHIZOPUS INOCULATIONS

The results with Rhizopus were less uniform than those wit Monilia. Even at the higher temperatures, the maturity of th fruit, the condition of the fungus, and other factors seemed to hav an important modifying influence, and at 15° C. and below th results were quite variable, especially as to the period of incubatio and percentage of infection.

The data for the Rhizopus needle inoculations on fruit that ha been previously cooled to approximately the temperature at whic it was to be stored[4] are shown in Figures 7 and 8, and the rate o growth for rots that had started before being placed at the variou temperatures is shown in Figure 9. The rate of increase in diamete at the various temperatures is shown in the smoothed curve *b* o Figure 2 and the incubation period in curve *c* of Figure 4.

It will be seen that the early development of Rhizopus was muc slower than that of Monilia, especially at the lower temperatures

[4] In an earlier report the inoculations were made on warm peaches. BROOKS, C., and COOLEY, J. S Op. cit.

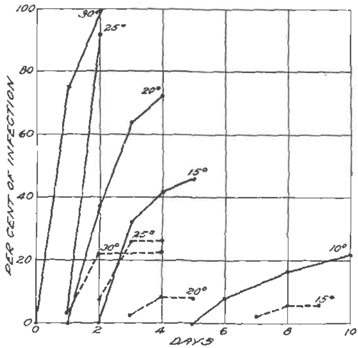

FIG. 8.—Temperature curves showing the percentage of infection with Rhizopus inoculations; solid lines, needle inoculations; broken lines, dusting inoculations on punctured peaches. With the needle inoculations there were variations from the average as great as 25 per cent at 30°, 25°, and 20° C. and as great as 100 per cent at 15° and 10°; and with the dusting inoculations there were variations from the average as great as 50 per cent at 30° and 25° and as great as 100 per cent at 20° and 15°. The variations were apparently due in part to differences in the maturity of the peaches. The needle-inoculation results are based on 12 series of experiments. (Fig. 7.) The dusting results are the average of 5 series of experiments, 1 with Hiley and 4 with Belle

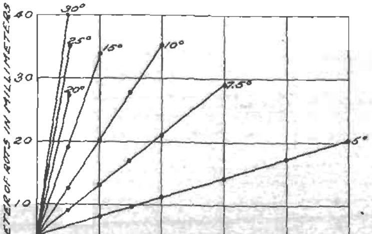

FIG. 9.—Rate of growth at different temperatures for Rhizopus rots that were already started, based on the results of 16 series of experiments at the higher temperatures and 5 series at 7.5° and 5° C. Some of the individual experiments varied from the average as much as 15 per cent. A part of this variation was apparently due to differences in the maturity of the fruit

but that when once established the rots enlarged more rapidly than those of Monilia at all of the higher temperatures, especially at 25° C. and above. Rhizopus was much more responsive to temperature changes than Monilia. It made a better development at 30° than at any other temperature tested, whereas this was above the optimum for Monilia. It was unable to start rots on peaches previously cooled to 7.5° and did not always produce them at 10°, but rots that had made a start at warmer temperatures continued to enlarge at 7.5° and even at 5°, but not at 2.5°. (Fig. 9.)

With Monilia needle inoculations, rots developed at practically 100 per cent of the punctures, and with Monilia dusting inoculations there was a very high percentage of infection at 10° C. and warmer and a gradual increase with time in the number of rots at 5° and 2.5°. (Fig. 5.) With Rhizopus, the infection from needle inoculations was not universal even at 25° and 30°, and at all lower temperatures it was very much poorer than with the Monilia dusting inoculations. (Figs. 5 and 8.) At 10° Rhizopus gave an average of only 22 per cent of infection 10 days after needle inoculation.

The infection from the Rhizopus dusting inoculations on punctured peaches was still poorer, never averaging more than 26 per cent even at the most favorable temperatures and being entirely lacking at 10° and colder. (Fig. 8.)

When once established, the Rhizopus rots enlarged at practically the same rate, regardless of the method of inoculation.

With Rhizopus, as already reported for Monilia, apparently sound peaches as well as the punctured ones were included in the dusting experiments. Rots occasionally developed on these dusted peaches, but not much more frequently than on the peaches that were not dusted. Apparently, germinating Rhizopus spores seldom if ever penetrate the sound skin of market-ripe peaches, and it is the opinion of the writers that the unbroken skin of the peach furnishes a complete protection against Rhizopus, except in cases where the sound fruit is in close contact with that which is already decayed.

DELAYS AT CONSTANT TEMPERATURES

In addition to the experiments in which the inoculated fruit was placed immediately at the temperature at which it was to be held, other tests were made in which it was delayed at a higher temperature before being placed at a lower one. The results are shown in Figures 10 to 17, inclusive.

APPLICATION OF EQUATED TEMPERATURE VALUES

So far as possible the results of the delays were interpreted in the light of the temperature studies previously reported and the actual growth compared with hypothetical growth curves developed from the temperature values of Figures 2 and 4.

As an illustration of the method of developing the hypothetical curves, the line c_1 of Figure 10 may be taken. After inoculation the peaches were held at 20° C. for 12 hours before storage at 7.5°. According to the values of Figure 4, 12 hours at 20° would allow Monilia to accomplish about 61 per cent of the work necessary to the production of a 5-mm. rot, leaving 39 per cent to be carried out at 7.5°, and according to the 7.5° values of the same figure 20 hours

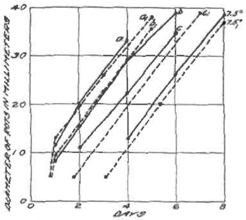

FIG. 10.—Effect of delay in cooling peaches that were needle inoculated with Monilia; the average of the results from 3 series of experiments, 1 each on Hiley, Belle, and Elberta. The solid lines give the actual results, the broken lines the estimated results based on the temperature values of Figures 2 and 4. Curves a and a_1, at 7.5° C. after 24 hours at 25°; b and b_1, at 7.5° after 24 hours at 20°; c and c_1, at 7.5° after 12 hours at 20°; also results at a constant temperature of 7.5°

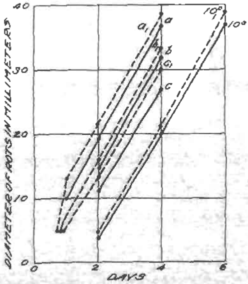

FIG. 11.—Effect of delay in cooling peaches that were needle inoculated with Monilia; the average of the results from 4 series of experiments, 1 each on Carman and Belle and 2 on Elberta. The solid lines give the actual results, the broken lines the estimated results based on the temperature values of Figures 2 and 4. Curves a and a_1, at 10° C. after 24 hours at 25°; b and b_1, at 10° after 22 hours at 20°; c and c_1, at 10° after 18 hours at 30°; also results at a constant temperature of 10°

would be required to complete the work at that temperature, making a total of 42 hours for the incubation period. After reaching the 5-mm. stage the rots would be expected to develop according to the growth values for 7.5°, as shown in Figure 2.

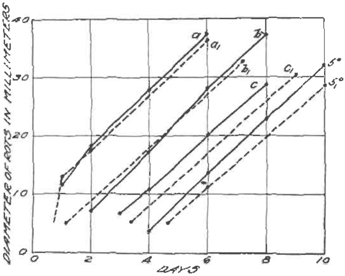

FIG. 12.—Effect of delay in cooling peaches that were needle inoculated with Monilia; the average of the results from 3 series of experiments, 1 on Hiley and 2 on Elberta. The solid lines give the actual results, the broken lines the estimated results based on the temperature values of Figures 2 and 4. Curves *a* and a_1, at 5° C. after 24 hours at 25°; *b* and b_1, at 5° after 18 hours at 30°; c and c_1, at 5° after 12 hours at 15°; also results at a constant temperature of 5°

A comparison of the hypothetical curves for the Monilia needle inoculations with the actual growth curves (figs. 10 to 12, inclusive) shows a fairly close agreement in most cases in both the incubation period and the rate of growth. In both the delayed and immediate-

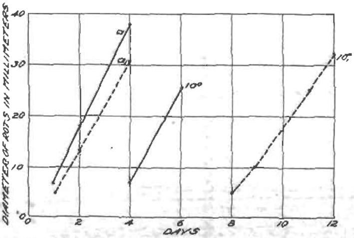

FIG. 13.—Effect of delay in cooling peaches that were needle inoculated with Rhizopus; the average of the results from 2 series of experiments, 1 each on Carman and Belle. The solid lines show the actual results, the broken lines the estimated results based on the temperature values of Figures 2 and 4. Curves *a* and a_1 at 10° C. after 18 hours at 25°. The peaches of the 10° curve were not cooled previous to inoculation

storage experiments at 10° C. the actual growth was behind the hypothetical; whereas at 7.5° and 5° the actual growth was usually ahead of the hypothetical, in three instances 8 to 15 hours ahead.

There would necessarily be a lag in temperature adjustment in moving the fruit from a higher to a lower temperature, and the

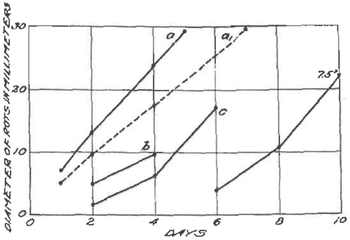

FIG. 14.—Effect of delay in cooling peaches that were needle inoculated with Rhizopus, the average of the results from three series of experiments on Belle peaches. The solid lines show the actual results, the broken line the estimated results based on the temperature values of Figures 2 and 4. Curves *a* and a_1, at 7.5° C. after 24 hours at 25°; *b*, at 5° after 18 hours at 25°; *c*, at 7.5° after 12 hours at 25°. As Rhizopus does not produce a rot when placed at once at 7.5° or 5°, the values of Figures 2 and 4 do not supply the data to estimate curves for *b* and *c*. The peaches of the 7.5° curve of Figure 14 were not previously cooled

fungus probably retains the stimulus of the higher temperature for a short time after removal to the lower, so it would be expected that

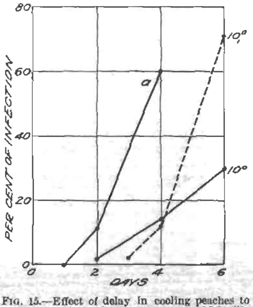

FIG. 15.—Effect of delay in cooling peaches to 10° C., as shown in the percentage of Monilia infection; the average of the results from 3 series of experiments, 1 each on Hiley, Belle, and Elberta. The peaches received 10 punctures each with No. 18 wire and were then dusted with Monilia spores. Curve *a* at 10° after 20 hours at 25°. The solid 10° line shows the results obtained in the present experiments; the broken line the average results of 16 tests as reported in Figure 5

the actual values might run somewhat ahead of the hypothetical, as developed by the methods described for Figures 2 and 4. The

differences between the hypothetical and the actual results, however, are little if any greater than the variation between the individual

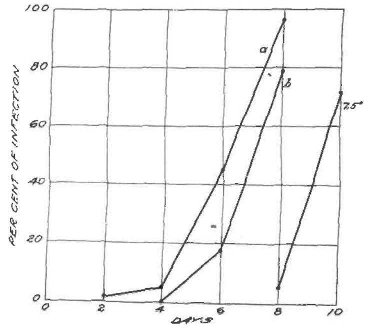

FIG. 16.—Effect of delay in cooling peaches, as shown in the percentage of Monilia infection, an experiment with Belle peaches. The peaches received 10 punctures each with No. 18 wire and were then dusted with Monilia spores. Curve *a*, at 7.5° C. after 24 hours at 20°; *b*, at 7.5° after 12 hours at 20°. The 7.5° graph shows results at that temperature held constant

experiments at constant temperatures as described in the legend for Figure 1.

In the case of the Rhizopus needle inoculations (figs. 13 and 14) the actual results were 15 to 30 hours ahead of the hypothetical.

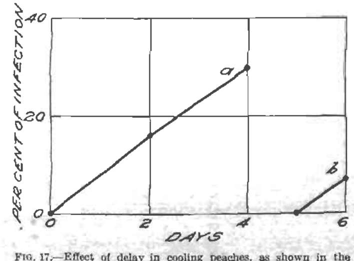

FIG. 17.—Effect of delay in cooling peaches, as shown in the percentage of Rhizopus infection, the average of the result from three series of experiments on Belle peaches. The peaches received 10 punctures with No. 18 wire and were then dusted with Rhizopus spores. Curve *a*, at 7.5° after 24 hours at 25°; *b*, at 7.5° after 12 hours at 25°. Warm inoculated peaches placed immediately at 7.5° did not develop rots

With the constant-temperature curves at 7.5° and 10° C., the contrast was much greater than this, but the peaches used in these experiments were not cooled before inoculating, whereas those upon which the

hypothetical values are based were cooled to approximately the storage temperature before inoculating. With the latter method no Rhizopus growth was obtained at 7.5°, and the growth at 10° was greatly delayed.

MONILIA NEEDLE INOCULATIONS

The effect of delay in cooling on Monilia needle inoculations is shown in Figures 10 to 12, inclusive. As compared with fruit stored immediately at 10° C., a delay of 24 hours at 25° put the rots about 44 hours ahead, a delay of 22 hours at 20° put them about 33 hours ahead, and a delay of 18 hours at 30° put them about 20 hours ahead.

As compared with immediate storage at 7.5° C., a delay of 24 hours at 25° gave the rots a lead of about 72 hours, a delay of 24 hours at 20° gave a lead of about 54 hours, and a delay of 12 hours at 20° gave a lead of about 34 hours.

As compared with immediate storage at 5° C., a delay of 24 hours at 25° gave the rots a lead of about 115 hours, a delay of 18 hours at 30° gave a lead of about 67 hours, and a delay of 12 hours at 15° gave a lead of about 33 hours.

MONILIA DUSTING INOCULATIONS

The effect of delay in cooling punctured peaches that were dusted with Monilia spores is shown in Figures 15 and 16. As compared with immediate storage at 10° C., a delay of 20 hours at 25° gave a lead of about 60 hours, as shown in the number of rots. As compared with immediate storage at 7.5° a delay of 24 hours at 20° gave a lead of about 88 hours, and a delay of 12 hours at 20° gave a lead of about 56 hours.

It will be noted that a given delay in cooling had much greater effect upon the dust inoculations than upon the needle inoculations as reported above, in fact about twice the effect. This is in agreement with the relative values reported in Figure 4.

RHIZOPUS INOCULATIONS

The effect of delay in cooling on Rhizopus needle inoculations is shown in Figures 13 and 14. Figure 17 shows the effect of delay in cooling dusting inoculations. A delay of 18 hours at 25° C. before storage at 10° accelerated needle inoculation rots about 76 hours. (Fig. 13.) A delay of 24 hours at 25° before storage at 7.5° caused an acceleration of about 150 hours, and a delay of 12 hours at 25° caused an acceleration of about 74 hours, as compared with warm peaches placed immediately at 7.5°. (Fig. 14.) Rhizopus rots did not develop at 7.5° when the peaches were cooled before inoculation. A delay of 18 hours at 25° before storage at 5° made it possible for the rots to appear at the 5° temperature. (Fig. 14.)

With peaches that were punctured and then dusted with Rhizopus spores, a delay of 24 hours at 25° C. before storing at 7.5° gave the rots a lead of five days over similar inoculations on peaches that were delayed but 12 hours at 25° before storing at 7.5°. (Fig. 17.)

A comparison of the results of Figure 17 with those of Figures 13 and 14 shows that delayed cooling had a very much greater effect upon Rhizopus dust inoculation than upon the needle inoculations. With both types of inoculation the effect of delayed cooling was much greater with Rhizopus than with Monilia.

DUPLICATION OF CAR-TEMPERATURE CONDITIONS

METHOD AND EQUIPMENT

The experiments previously reported were conducted in a series of six refrigerator boxes, each having a capacity of about 1 cubic yard. Each refrigerator box was supplied with a continuous and controlled stream of brine that was held at an approximately constant temperature. It was found that by setting the brine control so that the box

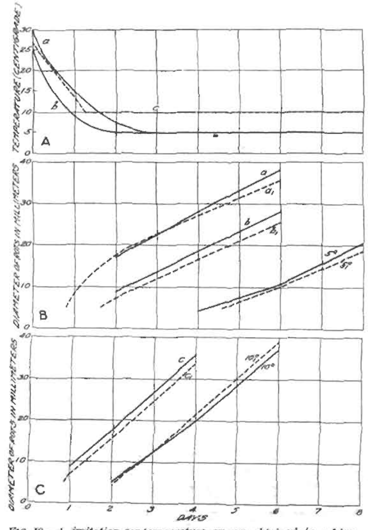

FIG. 18.—A, imitation car-temperature curves obtained in refrigerator boxes (see p. 522). B, growth curves for Monilia (needle inoculations) on Belle peaches at the temperatures shown in the curves of A and also at a constant temperature of 5° C. The solid lines give the actual results and the broken lines the estimated results based on the temperature values of Figures 2 and 4. Curves *a* and a_1, the rate of growth at the temperatures of curve A, *a*; *b* and b_1, at the temperatures of curve A, *b*. C, as B, but showing the growth at the temperatures of curve A, *c*, and also at a constant temperature of 10°

would be cooled to a temperature such as a car of fruit might have upon arrival at destination and then placing jars of hot water in the box a gradually falling temperature curve could be obtained similar to that of refrigerator cars. The initial temperature of the box was determined by the initial temperature of the water and the rate of cooling by the quantity of water used. The peaches were held at a distance from both the brine coils and the hot water, and the air of the box was kept in circulation by means of a slow-moving fan.

Complete temperature records were obtained by means of thermographs and standard thermometers placed beside the peaches. The temperature curves thus obtained are shown at A in Figures 18 and 19.

In addition to the car-temperature conditions, constant temperatures were maintained at the same time in other boxes of the series.

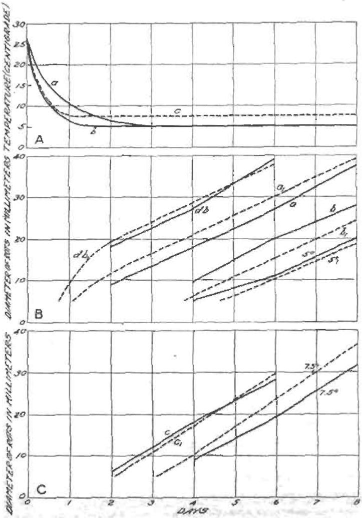

FIG. 19.—A, imitation car-temperature curves obtained in refrigerator boxes. B, growth curves for Monilia (needle inoculations) on Elberta peaches under the temperature conditions shown in curves A, *a*, and A, *b*, and also at a constant temperature of 5° C. The solid lines show the actual results and the broken lines the estimated results based on the temperature values of Figures 2 and 4. Curves *a* and a_1, rate of growth at the temperature of curve A, *a*; *b* and b_1, at the temperature of curve A, *b*; *db* and db_1, the rate of growth on peaches that were delayed at 26° for 24 hours before storing under the conditions shown in curve A, *b*. C, as B, but showing the growth of Monilia at the temperatures of curve A, *c*, and also at a constant temperature of 7.5°

APPLICATION OF EQUATED TEMPERATURE VALUES

The above conditions made it possible to hold part of a particular lot of inoculated peaches at a constant temperature while the others of the same lot were exposed to conditions similar to those that prevail in a refrigerator car. The results are shown in Figures 18, B and C, and 19, B and C. The solid lines show the actual growth and the broken lines the estimated growth based on the values of Figures 2 and 4.

The curves for the estimated growth were developed as described under "Delays at constant temperatures" (p. 516), except that instead of having two temperatures to consider there was often a different temperature for every hour. Curve a_1 of Figure 18, B, may be taken as an illustration. If the temperatures shown in the various hour periods of curve *a*, Figure 18, A, are converted into work units according to the values of Figure 4, it is found that 19 hours would be required for the Monilia rots to pass the incubation period and reach a diameter of 5 mm. They should then enlarge at a rate to be determined by converting the succeeding temperature values of curve *a*, Figure 18, A, into growth values according to the scale of Figure 2. The result is a gradually changing curve as shown in curve a_1 of Figure 18, B.

A comparison of the hypothetical Monilia curves with the actual growth curves shows practically as close agreement as was found with delays at constant temperatures. (Figs. 10 to 17, inclusive.) In five instances the actual growth was ahead of the hypothetical, in three instances behind it, and in three cases almost identical with it.

EFFECTS OF DELAYED COOLING

As compared with storing the Monilia-inoculated fruit immediately at 5° C., holding it at the temperature of curve *a*, Figure 18, A, gave the rots a lead of 130 hours; holding it at the temperature of curve *b*, Figure 18, A, or curve *a*, Figure 19, A, gave a lead of about 85 hours; holding it at the temperature of curve *b*, Figure 19, A, gave a lead of about 35 hours; and holding it at 26° for 24 hours and then at the temperature of curve *b*, Figure 19, A, gave a lead of about 135 hours. (Figs. 18, B, and 19, B.) As compared with storing inoculated fruit immediately at 7.5° C., holding it at the temperature of curve *c*, Figure 19, A, gave the rots a lead of about 38 hours. (Fig. 19, C.) As compared with storing it immediately at 10°, holding it at the temperature of curve *c*, Figure 18, A, gave the rots a lead of about 42 hours. (Fig. 18, C.)

The above accelerations in the time of the appearance of the rots resulting from the moderate delays shown in the curves of Figures 18, A, and 19, A, give evidence of the extreme importance of prompt loading and rapid cooling of harvested peaches.

PEACH ROTS IN TRANSIT

Experiments were also made in the standard refrigerator cars under commercial shipping conditions. The work was conducted in the years 1921 to 1926, inclusive, and included a record on 13 different refrigerator cars. The temperatures of Figure 20, A, are for cars shipped from Cornelia, Ga., to Chicago, Ill., but all other records are on shipments from Fort Valley, Ga., to New York City. The car-temperature curves are shown in Figures 20, A, to 30, A, inclusive.

METHODS AND EQUIPMENT

Except where otherwise stated, the cars were loaded four layers high with six-basket carriers, and the experimental peaches were placed in the middle of the stack, halfway between the bunker and the door. Half of the experimental lot was placed in the top layer and half in the bottom layer.

A thermograph was placed with each lot of peaches. It was fastened in the middle of the package, and peaches were packed around and in contact with it. It is believed that this arrangement gave the instrument practically the same temperature as the surface flesh of the fruit. The thermographs were tested before and after

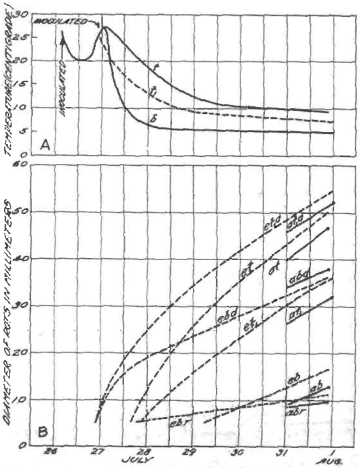

FIG. 20.—A, curves showing the temperature of peaches in two refrigerator cars that were loaded at approximately the same time, one with crates, the other with bushel baskets; also peach temperatures before loading. Curve *t*, fifth and top layer of crates in the fourth stack from the bunker; *b*, bottom layer of the same stack; t_1, third and top layer of baskets in the sixth stack from the bunker. B, development of rots (needle inoculations) on well-matured Elberta peaches under the temperature conditions shown in A. The position of the heavy dots at the right of the figure shows the actual size of the rots at the destination, and the lines connecting with these trace the growth movement backward according to the values of Figure 2. The broken-line curves give the estimated incubation and growth based on the values of Figures 2 and 4: Curves *et* and *at* give the results under the conditions of curve A, *t*, with Monilia inoculations 3 hours before loading; *eb* and *ab* under the conditions of curve A, *b*, with Monilia inoculations 3 hours before loading; *ebd* and *abd* under the conditions of curve A, *b*, with Monilia inoculations 22 hours before loading; et_1 and at_1 under the conditions of curve A, t_1, with Monilia inoculations at loading; et_1d and at_1d under the conditions of curve A, t_1 with Monilia inoculations 19 hours before loading; *ebr* and *abr* under the conditions of curve A, *b*, with Rhizopus inoculations 22 hours before loading

the shipment, and, as a further check on the instruments, the temperature of the peaches was always taken on arrival at destination. It is believed that with these various precautions a fairly accurate temperature record was obtained.

The inoculations were made as previously described (p. 507) except that 40 or more peaches were used under each condition

APPLICATION OF EQUATED TEMPERATURE VALUES

With peaches in transit, a record of the actual development of the rots could be obtained only at the end of the shipment. But with the temperatures known it was possible to plot hypothetical growth curves according to the values of Figures 2 and 4, as described for the

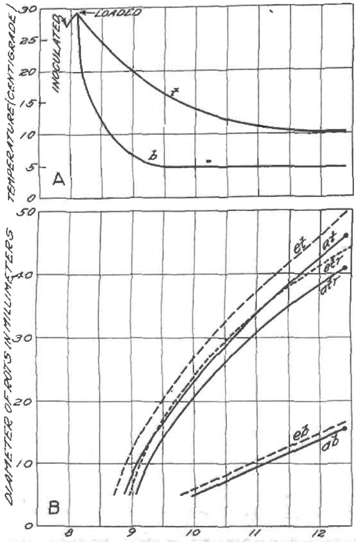

FIG. 21.—A, curves showing the temperature of peaches in a refrigerator car in transit and also the temperature before loading. Curve *t*, fourth and top layer of peach crates in the sixth stack from the bunker; *b*, bottom layer two stacks from the bunker. B, development of Monilia (needle inoculations) on well-matured Elberta peaches under the temperature conditions shown in A. The average size of the rots at the destination is shown by the position of the heavy dots at the right of the figure, *at* and *atr* for the top of the car and *ab* for the bottom. The solid lines connecting with these dots attempt to trace the growth curves backward by applying the growth values of Figure 2 to curves *t* and *b* of A. Curves *et*, *etr*, and *eb* give the estimated results under the conditions shown in A, based on the incubation values of Figure 4 and the growth values of Figure 2. Curves *etr* and *atr* refer to Rhizopus, the others to Monilia. All inoculations were made approximately four hours before loading

imitation car temperatures (p. 524), and it was also possible to trace the probable line of growth backward, with the size of the rots at the end of the shipment as a starting point. The hypothetical growth curves thus developed for the Monilia needle inoculations and a part of those for the Rhizopus needle inoculations are shown in Figures 20 to 30, inclusive.

Monilia Needle Inoculations

A study of the results of the Monilia needle inoculations shows that there were 11 instances in which the actual growth of the rots was ahead of the estimated growth and 29 instances in which it was behind. In 19 cases, however, there was not more than four hours' difference in the two. If these are excluded, only 2 instances remain in which the rots were ahead of the estimated growth and 19 instances in which they were 5 to 38 hours behind it. (See "Varietal resistance," p. 529.)

As shown in Figure 4 and emphasized in later paragraphs, needle-inoculation rots make a more rapid development than other types of Monilia infection. It is therefore evident from the above results that with the temperature of the fruit known, the incubation and growth can be plotted according to the values of Figures 2 and 4 with the expectation that there will be but few, if any, instances in which the actual growth will be ahead of that which has been estimated.

Monilia Dusting Inoculations

The results from the Monilia dusting inoculations are shown in Table 3. The rots average about one-third the size of the needle-inoculation rots exposed to the same conditions, which is approximately the ratio that holds in a comparison of Figures 1 and 6. The temperature values of Figures 4 and 6 do not form a basis for more than an approximate estimate on the results from dusting inoculations, but it can be said that in general the shipping results agree with the values shown in these figures. There are more instances in which the rots fall behind the expected results than exceed them.

Table 2.—*Development of Rhizopus rots on peaches in transit; needle inoculations*

Variety of peach	Temperatures as shown in—	Time of inoculation previous to loading (hours)	Condition upon arrival					
			Percentage of peaches affected		Average diameters of rots (millimeters)			
					Top of car		Bottom of car	
			Top of car	Bottom of car	Actual	Estimated [a]	Actual	Estimated [a]
Elberta	Fig. 21, A	4	....	....	41	44	0.2	0
Do	Fig. 20, A	22	....	....	Total.	Total.	9.5	11
Do	do	3	....	....	51	38	0	0
Yellow Hiley	Fig. 22, A	22	....	....	44.5	39	1.4	0
Do	do	6	....	....	18.4	21	0	0
Do	Fig. 28, A	5	....	....	.6	0	0	0
Elberta	Fig. 29, A	2	....	....	0	0	0	0
Hiley	Fig. 23, A	17	90	100	54.3	45	2.3	0–9
Do	do	2	30	0	6.3	28	....	....
Do	Fig. 24, A	16–42	100	90	70.7	49	39.5	21
Do	do	6–32	100	70	42	37	15.0	5–10
Elberta	Fig. 25, A	14	50	20	29.2	32	3.1	0
Do	do	2	70	0	11.3	15	....	....
Do	Fig. 26, A	16	80	50	15.4	46	3.8	11
Do	do	4	60	10	13.6	30	3.2	0

[a] Estimated on the basis of the values of Figures 2 and 4.

Rhizopus Inoculations

The results from the Rhizopus needle inoculations are shown in Table 2 and in Figures 20 to 25. In 6 instances the growth was

greater than would be expected from the values of Figures 2 and 4, in 4 instances it was less, and in 18 instances it was the same or prac-

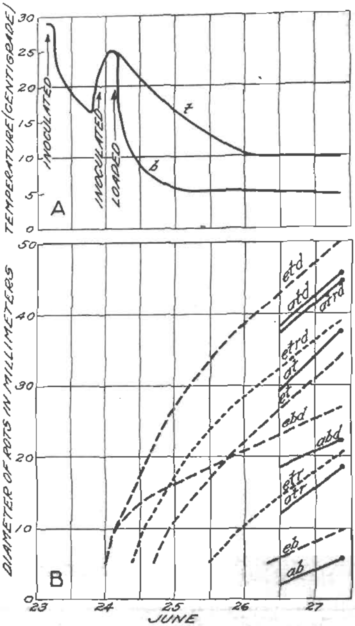

FIG. 22.—A, curves showing the temperature of peaches in a refrigerator car in transit and also the temperatures before loading. Curve *t*, fourth and top layer; *b*, bottom layer in the fourth stack of crates from the bunker. B, development of rots (needle inoculations) on Yellow Hiley peaches under the temperature conditions shown in A. The position of the heavy dots at the right of the figure shows the actua size of the rots at the destination, and the lines connecting with these trace the growth movement backward according to the values of Figure 2. The broken-line curves give the estimated incubation and growth based on the values of Figures 2 and 4: Curves *et* and *at* give the results under the conditions of, curve A, *t*, with Monilia inoculations 6 hours before loading; *etd* and *atd*, under the conditions of curve A, *t*, with Monilia inoculations 24 hours before loading; *eb* and *ab* under the conditions of curve A, *b*, with Monilia inoculations 6 hours before loading; *ebd* and *abd*, under the conditions of curve A, *b*, with Monilia inoculations 24 hours before loading; *etr* and *atr* under the conditions of, curve A, *t*, with Rhizopus inoculations 6 hours before loading; *etrd* and *atrd* under the conditions of curve A, *t*, with Rhizopus inoculations 24 hours before loading

tically the same; 10 of the 18 instances, however, were where both the actual and estimated values were zero or approximately zero.

As a whole, the results indicate that the Rhizopus values of Figures 2 to 4 represent about the average of what can be expected; but the rather wide variations from this average, in some instances, give further emphasis to the erratic nature of Rhizopus rots (p. 514).

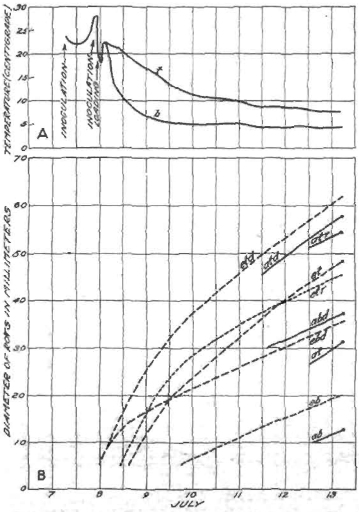

FIG. 23.—A, curves showing the temperature of peaches in a refrigerator car in transit and also the temperatures before loading. Curve *t*, fourth and top layer of crates in the fourth stack from the bunker; *b*, bottom layer in the same stack. B, development of rots (needle inoculations) on Hiley peaches under the temperature conditions shown in A. The position of the heavy dots at the right of the figure shows the actual size of the rots at the destination, and the lines connecting with these trace the growth movement backward according to the values of Figure 2. The broken-line curves show the estimated incubation and growth based on the values of Figures 2 and 4: Curves *et* and *at* give the results under the conditions of curve A, *t*, with Monilia inoculations 2 hours before loading; *etd* and *atd*, under the conditions of curve A, *t*, with Monilia inoculations 17 hours before loading; *eb* and *ab*, under the conditions of curve A, *b*, with Monilia inoculations 2 hours before loading; *ebd* and *abd*, under the conditions of curve A, *b*, with Monilia inoculations 17 hours before loading; *etr* and *atr*, under the conditions of curve A, *t*, with Rhizopus inoculations 17 hours before loading

VARIETAL RESISTANCE

A study of the tables and figures shows that with Monilia needle inoculations on Elberta peaches the actual growth was behind the estimated in 13 instances, ahead of it in none, and approximately the same in 9. With similar inoculations on Hiley peaches the actual growth was behind the estimated in 5 instances, ahead of it in 2, and

approximately the same in 11. The actual growth of Monilia on Elberta thus ran distinctly behind the estimated in 59 per cent of the tests and ahead in none. While on Hiley it ran definitely behind in only 28 per cent and ran ahead in 11 per cent.

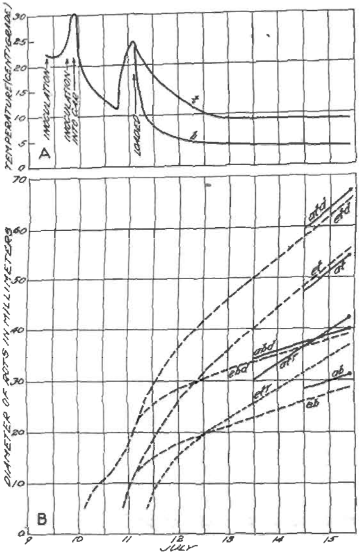

FIG. 24.—A, curves showing the temperature of peaches in a refrigerator car in transit and also the temperatures before loading. Curve *t*, fourth and top layer of crates in the fourth stack from the bunker; *b*, bottom layer in the same stack. B, development of rots (needle inoculations) on Hiley peaches under the temperature conditions shown in A. The position of the heavy dots at the right of the figure shows the actual size of the rots at the destination and the lines connecting with these trace the growth movement backward according to the values of Figure 2. The broken-line curves show the estimated incubation and growth based on the values of Figures 2 and 4: Curves *et* and *at* give the results under conditions of curve A, *t*, with Monilia inoculations 6 hours before cooling; *etd* and *atd*, under the conditions of curve A, *t*, with Monilia inoculations 16 hours before cooling; *eb* and *ab*, under the conditions of curve A, *b*, with Monilia inoculations 6 hours before cooling; *ebd* and *abd*, under the conditions of curve A, *b*, with Monilia inoculations 16 hours before cooling; *etr* and *atr*, under the conditions of curve A, *t*, with Rhizopus inoculations 6 hours before cooling

With Rhizopus needle inoculations on Elberta peaches the actual rowth was behind the estimated in 3 instances, ahead of it in 1, and pproximately the same in 11. With similar inoculations on Hiley eaches the actual growth was behind the estimated in 1 instance,

ahead in 5, and approximately the same in 7. On a percentage basis, the actual results were behind the estimated in 20 per cent of

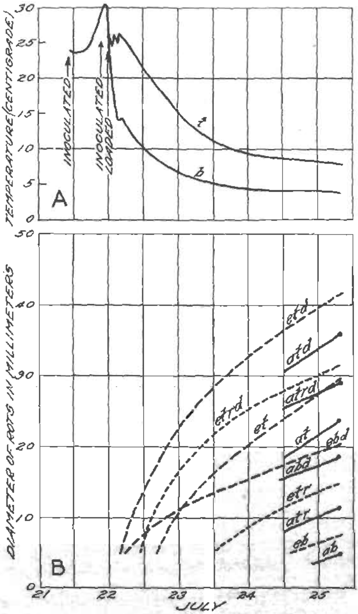

FIG. 25.—A, curves showing the temperature of peaches in a refrigerator car in transit and also the temperatures before loading. Curve *t*, fourth and top layer of crates in the fourth stack from the bunker; *b*, bottom layer of crates in the same stack. B, development of rots (needle inoculations) on Elberta peaches under the temperature conditions shown in A. The position of the heavy dots at the right of the figure shows the actual size of the rots at the destination, and the lines connecting with these trace the growth movement backward according to the values of Figure 2. The broken-line curves show the estimated incubation and growth based on the values of Figures 2 and 4: Curves *et* and *at* give the results under conditions of curve A, *t*, with Monilia inoculations 2 hours before loading; *etd* and *atd*, under the conditions of curve A, *t*, with Monilia inoculations 14 hours before loading; *eb* and *ab*, under the conditions of curve A, *b*, with Monilia inoculations 2 hours before loading; *ebd* and *abd*, under the conditions of curve A, *b*, with Monilia inoculations 14 hours before loading; *etr* and *atr*, under the conditions of curve A, *t*, with Rhizopus inoculations 2 hours before loading; *etrd* and *atrd*, under the conditions of curve A, *t*, with Rhizopus inoculations 14 hours before loading

the tests with Elberta and in 8 per cent with Hiley; and they were ahead of the estimated in 7 per cent of the tests with Elberta and 38

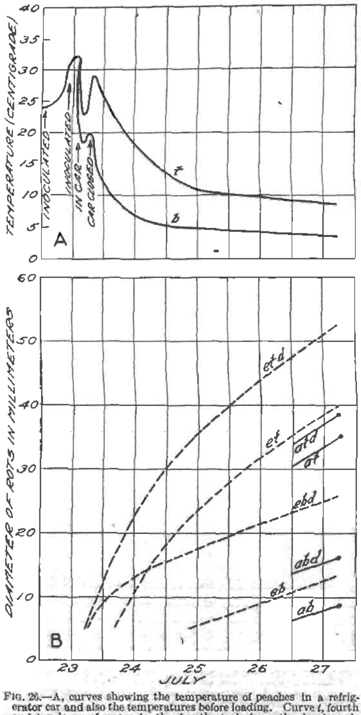

FIG. 26.—A, curves showing the temperature of peaches in a refrigerator car and also the temperatures before loading. Curve *t*, fourth and top layer of crates in the fourth stack from the bunker; *b*, bottom layer of crates in the same stack. B, development of Monilia (needle inoculations) on rather green Elberta peaches under the temperature conditions shown in A. The position of the heavy dots at the right of the figure shows the actual size of the rots at the destination, and the lines connecting with these trace the growth movement backward according to the values of Figure 2. The broken-line curves show the estimated incubation and growth based on the values of Figures 2 and 4: Curves *et* and *at* give the results under the conditions of curve A, *t*, with inoculations 4 hours before loading; *etd* and *atd*, under the conditon of curve A, *t*, with inoculations 16 hours before loading; *eb* and *ab*, under the conditions of curve A, *b*, with inoculations 4 hours before loading; *ebd* and *abd*, under the conditions of curve A, *b*, with inoculations 16 hours before loading

The results indicate that Elberta peaches are considerably more resistant than Hiley to both Monilia and Rhizopus rots. This is in agreement with market reports and with general opinion. It should be noted, however, that the data for the shipment described in Figure 26 account for much of the resistance to rot shown by the Elberta variety and that the peaches of this shipment were rather green. It was noticed in a number of experiments that greenness seemed to introduce a greater factor of rot resistance with Elberta than with other varieties. In the present studies Elberta peaches in a market-ripe condition and with a broken skin have shown but little more resistance to rot than Hiley, Belle, or Carman. It is the opinion of the writers that the greater freedom from rot found in shipments of well-matured Elberta peaches is largely due to a greater resistance to punctures and bruises.

TYPES OF INOCULATION

Several different types of inoculation with Monilia were tested. The results from the needle inoculations are shown in Figures 20 to 30, inclusive, and the results from other methods in Table 3.

TABLE 3.—*Effect of different types of Monilia inoculation on peaches shipped under different temperature conditions*

Treatment: Nature	Treatment: Time before loading (hours)	Percentage of peaches affected: Top of car	Percentage of peaches affected: Bottom of car	Average diameters of rots (millimeters): Top of car	Average diameters of rots (millimeters): Bottom of car	Average number of rots per peach: Top of car	Average number of rots per peach: Bottom of car
HILEY PEACHES SHIPPED UNDER THE TEMPERATURE CONDITIONS SHOWN IN FIGURE 23							
No inoculation		4.8	2.2	22.6	25	0.05	0.02
Rubbed (not smeared) with rotten peaches carrying but few spores	17	100	95.3	Total.	68		
Do	2	100	26.7	45.6	12.4		
Dusted with spores (no punctures)	17	87.5	13.9	51.2	32.3		
Do	2	37.8	10.7	17.0	18.2		
Dusted with spores after receiving 20 pin-point punctures to the peach	17	97.3	64.7	13.8	4.6	4.3	1.4
Do	2	84.2	9.1	3.1	2.3	2.3	.1
Wet peaches punctured and dusted as above	17	100	92.5	Total.	4.8	(a)	7.1
Do	2	100	38.5	17.3	1.7	3.8	.9
HILEY PEACHES SHIPPED UNDER THE TEMPERATURE CONDITIONS SHOWN IN FIGURE 24							
No inoculation		7.1	2.8	22.8	14.9	0.08	0.03
Rubbed (not smeared) with rotten peaches carrying but few spores	6–32	80	52.9	37.8	20.7		
As above, but with peaches that had received 20 pin-point punctures to the peach	6–32	100	100	Total.	31.8		
Dusted with spores (no punctures)	16–42	30	29	23.8	19.8		
Do	6–32	32.4	2	24.8	58.0		
Dusted with spores after receiving 20 pin-point punctures to the peach	16–42	100	73.8	67.4	30.4	(a)	1.6
Do	6–32	97.4	76.9	10.8	7.6	3.5	2.4
Wet peaches punctured and dusted as above	16–42	100	100	Total.	58.8	(a)	(a)
Do	6–32	90	100	Total.	6.8	(a)	6.5
ELBERTA PEACHES SHIPPED UNDER THE TEMPERATURE CONDITIONS SHOWN IN FIGURE 25							
No inoculation		1.1	0.9	28.8	22.5	0.01	0.01
Rubbed (not smeared) with rotten peaches carrying no evident spores	2	96.7	13.3[a]	18.6	8.9	2.0	.1
Dusted with spores (no punctures)	20	59.3	0	12.3	0	.9	0
Do	2	37.5	0	7.4	0	.8	0
Dusted with spores after receiving 10 pin-point punctures to the peach	20	95.7	33.2	10.5	8.1	3.1	.6
Do	14	61.3	9.5	9.0	6.8	.9	.1
Do	2	38.6	3.0	6.3	6.2	.5	.03

TABLE 3.—*Effect of different types of Monilia inoculation on peaches shipped under different temperature conditions*—Continued

ELBERTA PEACHES SHIPPED UNDER THE TEMPERATURE CONDITIONS SHOWN IN FIGURE 26

Treatment: Nature	Treatment: Time before loading (hours)	Percentage of peaches affected: Top of car	Percentage of peaches affected: Bottom of car	Average diameters of rots (millimeters): Top of car	Average diameters of rots (millimeters): Bottom of car	Average of number of rots per peach: Top of car	Average of number of rots per peach: Bottom of car
No inoculation		2.6	0	21.5	0	0.03	0
Rubbed (not smeared) with rotten peaches carrying few if any spores	4	31.8	7.4			.6	.1
Dusted with spores (no punctures)	21	23.3	4.8	24.3	12.0	.3	.05
Do	15	6.9	3.5	8.6	11.8	.1	.05
Dusted with spores after receiving 10 pin-point punctures to the peach	26	71.9	46.4	17.5	10.2	1.4	1.0
Do	21	44.5	30.0	12.6	4.5	.7	.5
Do	15	85.2	10.5	18.4	10.6	2.6	.1
Do	4	16.7	0	13.2	0	.2	0 [a]

HILEY PEACHES SHIPPED UNDER THE TEMPERATURE CONDITIONS SHOWN IN FIGURE 30

Treatment: Nature	Treatment: Time before loading (hours)	Percentage of peaches affected: Top of car	Percentage of peaches affected: Bottom of car	Average diameters of rots (millimeters): Top of car	Average diameters of rots (millimeters): Bottom of car	Average of number of rots per peach: Top of car	Average of number of rots per peach: Bottom of car
CAR 1							
No inoculation		6	0	26.6	0	0.085	0
Dusted with spores after receiving 10 punctures each with No. 18 wire	2	100	7	15.3	16.0	5.9	.14
As above, but receiving 10 pin-point punctures each	2	95	12	17.8	8.7	4.2	.24
CAR 2							
No inoculation		8	1	32.0	22.5	.15	.04
Dusted with spores after receiving 10 punctures each with No. 18 wire	2	100	100	Total.	11.0	Total.	6.4
As above, but receiving 10 pin-point punctures each	2	100	78	Total.	12.7	Total.	2.3

[a] Rots confluent.

Needle inoculations gave practically 100 per cent infection in all cases. Of all the methods used they gave the largest rots at the end of the shipment thus indicating the shortest period of incubation.

Rubbing peaches with rotten fruit that carried few if any spores produced a high percentage of infection and a rapid rot, showing the serious results that may follow from allowing partly rotten peaches to come in contact with those that are intended for market.

Wetting the peaches before puncturing and dusting greatly increased both the percentage of infection and the size of the rots. This may have been partly due to a greater number of spores clinging to the wet fruit than to the dry, but the wetness probably also hastened germination and facilitated infection.

The rots on the peaches that were punctured and dusted without wetting averaged about one-third the size of the needle-inoculation rots, and as estimated by the growth values of Figure 2 were 2.5 to 3 days behind them in development.

The lead of the rots on peaches that were punctured before dusting over those that were dusted without intentional puncturing was in the number of the rots and not in their size. The same thing holds true in a comparison of the two types of dusted peaches with the

untreated checks. In fact the rots resulting from dry-dust inoculations a few hours before loading averaged much smaller than those on the untreated fruit. This difference was evidently due to the fact that most of the rots on the untreated peaches had made an earlier start than those on the dusted peaches.

The peaches that were punctured and dusted had an average of about four and five-tenths times as many rots as those which were dusted without puncturing and more than one hundred and sixty times as many as those which were untreated.

The results as a whole show that spores carried on the surface of the fruit may be a cause of serious loss in transit, especially if the peaches have been punctured or otherwise roughly handled.

Spores were rarely produced by the rots during the transit period, even when the needle-inoculation fruit was delayed in loading, and it is safe to state that they were never produced in time to develop rots before the arrival of the shipment at its destination. All of the rots developed, therefore, were produced by the spores or mycelium carried by the fruit at the time of loading.

SPRAYED AND UNSPRAYED FRUIT

The peaches used in all the previous experiments had been sprayed according to prevailing orchard schedules, but the writers were fortunate in being able to obtain comparable lots of sprayed and unsprayed fruit from the experimental plots of John W. Roberts and John C. Dunegan at Fort Valley, Ga. The peaches described as unsprayed had received no fungicidal treatment whereas the sprayed fruit had been given the full spray-schedule treatment. The peaches were picked and packed under commercial conditions, apparently sound peaches only being included in the pack. One to three crates of peaches were used under each shipping condition. The results are shown in Table 4.

TABLE 4.—*Percentage of sprayed and unsprayed peaches affected with Monilia rot in transit*

[All fruit was apparently sound at the time of packing]

Variety	Year	Temperature as shown in—	Percentage of peaches affected with Monilia rot at the time of unloading			
			Top		Bottom	
			Sprayed	Un-sprayed	Sprayed	Un-sprayed
Yellow Hiley	1922		13.3	32.8	5.0	13.1
Hiley	1921		10.0	13.5	1.6	2.8
Elberta	1921	Fig. 21, A	27.3	91.1	5.3	39.9
Yellow Hiley	1922	Fig. 22, A	4.6	26.4	2.2	12.5
Do.	1923	Fig. 27, A	11.8	31.7	6.3	12.5
Do.	1923	Fig. 28, A	1.7	21.4	.5	7.9
Elberta	1923	Fig. 29, A	1.3	3.7	0	0
Hiley	1924	Fig. 23, A	4.8	16.7	2.2	11.7
Do.	1924	Fig. 24, A	7.1	37.3	2.8	25.2
Elberta	1924	Fig. 25, A	1.1	12.0	.9	9.1
Do.	1924	Fig. 26, A	2.6	42.2	0	13.1
Average			7.8	29.9	2.5	13.4

The unsprayed peaches developed far more brown rot in transit than the sprayed ones, in many cases eight to ten times as much. The average for 11 cars showed 7.8 per cent of the sprayed fruit in the top of the cars affected with Monilia rot, as compared with 29.9 per cent of the unsprayed fruit; and 2.5 per cent of the sprayed fruit in the bottom of the car was similarly affected, as compared with 13.4 per cent of the unsprayed fruit. The results show that the value of orchard spraying does not end in the orchard, but follows the fruit to its final destination.

In the top of the car the unsprayed peaches had practically the same percentage of rot as sprayed peaches that had been dusted (not punctured) with Monilia spores a few hours before loading, but in the bottom of the car the unsprayed peaches had more than three times as many rots as the comparable lots of dusted peaches. (Tables 3 and 4.) This contrast between the top and the bottom of the cars might be explained on the hypothesis that at the time of loading the unsprayed peaches carried fewer spores but more incipient rots than sprayed peaches which were dusted with Monilia spores; the temperature of the bottom of the car causing a greater relative decrease in infection than in the development of rots that had already made a start.

The results of the various experiments emphasize the importance of having the peaches as free as possible from spores as well as infections at the time they are loaded for shipment.

DELAYS BEFORE LOADING

A study of the results from Monilia needle inoculations (figs. 20 to 27, inclusive) shows that with this type of infection every hour of delay before loading puts the rots in the top of the car more than three hours ahead and those in the bottom of the car more than five hours ahead.

A similar study of the Rhizopus needle inoculations (Table 2, interpreted by Figures 2 and 4) shows that every hour of delay before loading put the rots in the top of the car nearly five hours ahead. The delays also greatly increased the percentage of infection. A delay of 10 to 15 hours sometimes meant three to five times as many infections, and in other cases it meant the difference between no infection and 20 to 100 per cent of infection.

The effect of delay upon the Monilia dusting inoculations was also significant, but not easy to summarize. A delay of a few hours before loading often meant two to three times as many rots at the end of the shipment, sometimes 10 to 12 times as many. Where there was little contrast in the number of rots, the effect of the delay was usually evident in their size, the rots on the delayed fruit often being several times as large as those on the fruit that was more promptly loaded.

COOLING OF THE CAR

A comparison of Figures 20 to 30, inclusive, shows considerable variation in the cooling of the different cars. Since the inoculations and the fruit in the different cars were not usually comparable, it is impossible to accurately interpret these differences in cooling in terms of actual rot development at the destination. A comparison can be made, however, on the basis of the values of Figures 2 and 4.

A study of the results reported in Figures 25 and 30 shows that the

at the time of loading, but that at the end of 24 hours, the top of the car described in Figure 25 was down to 15° C. while the top of one of the cars described in Figure 30 had reached only 22.5°. According to the values of Figures 2 and 4 this difference in temperature meant that the rots in the top of one car were developing nearly

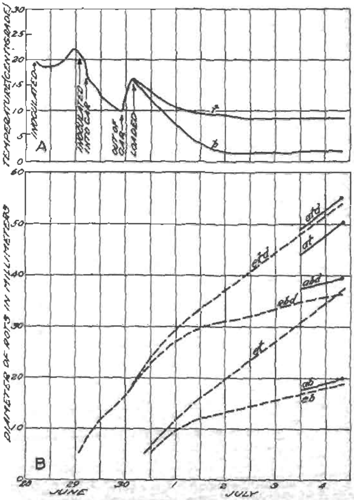

FIG. 27.—A, curves showing the temperature of peaches in a refrigerator car in transit and also the temperatures before loading. The experimental peaches were held inside the refrigerator car the night previous to final loading, and the temperature curves probably do not show the condition of the general load. Curve *t*, fourth and top layer in the fourth stack of crates from the bunker; *b*, bottom layer of the same stack. B, development of Monilia (needle inoculations) on Yellow Hiley peaches under the temperature conditions shown in A. The position of the heavy dots at the right of the figure shows the actual size of the rots at the destination, and the lines connecting with these trace the growth movement backward according to the values of Figure 2. The broken-line curves show the estimated incubation and growth based on the values of Figures 2 and 4: Curves *et* and *at* give the results under the conditions of curve A, *t*, with inoculations 3 hours before cooling; *etd* and *atd*, under the conditions of curve A, *t*, with inoculations 24 hours before cooling; *eb* and *ab*, under the conditions of curve A, *b*, with inoculations 3 hours before cooling; *ebd* and *abd*, under the conditions of curve A, *b*, with inoculations 24 hours before cooling

twice as rapidly as those in the other. In the shipments described in Figures 24, 27, and 28, the temperature in the top of the car at the end of 24 hours was still lower than that of Figure 25, indicating a still greater check in the development of rots.

The most interesting contrasts in cooling, however, are to be found

CONTRAST BETWEEN THE TOP AND THE BOTTOM OF THE CAR[1]

If the temperature curves of Figures 20 to 26 and 28 to 30, inclusive, are interpreted in terms of the work values or time-temperature units

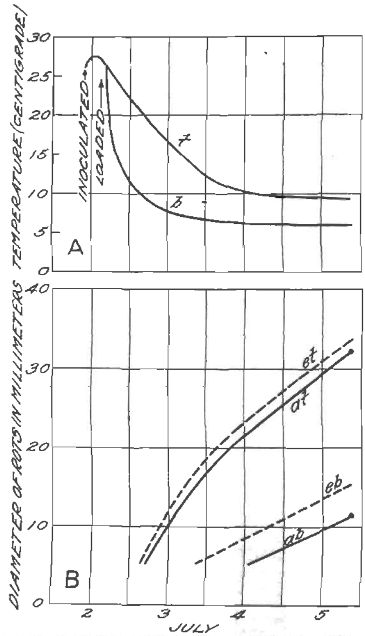

FIG. 26.—A, curves showing the temperature of peaches in a refrigerator car in transit and also the temperatures before loading. Curve *t*, fourth and top layer in the fourth stack of crates from the bunker; *b*, bottom layer of the same stack. B, development of Monilia (needle inoculations) on Yellow Hiley peaches under the temperature conditions shown in A. The position of the heavy dots at the right of the figure shows the actual size of the rots at the destination, and the lines connecting with these trace the growth movement backward according to the values of Figure 2. The broken-line curves show the estimated incubation and growth based on the values of Figures 2 and 4. Curves *et* and *at* give the results under the conditions of curve *t*; *eb* and *ab* under the conditions of curve *b*. All inoculations were made five hours before loading

of Figure 4 with the development of a 5-mm. rot represented by 100, it is found that for Monilia needle inoculations the temperatures

[1] Reference is here made to cars loaded four layers high with six-basket carriers.

recorded for the bottom of the cars would have an average rating of 105, whereas those for the top of the cars would have an average rating of 266, or two and fifty-three hundredths times as high as that for the bottom of the cars. If instead of the needle-inoculation curve, the Monilia dusting and puncture curve is used, the ratings are about 53 for the bottom of the cars and 133 for the top of the cars. These ratings mean that in the bottom of the car Monilia spores pushed into

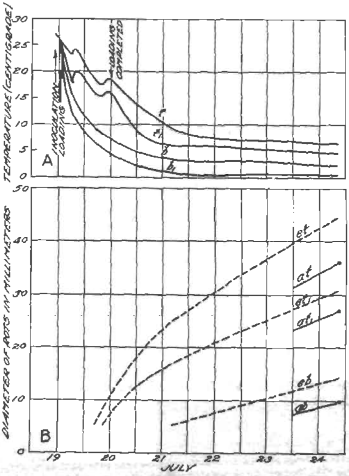

FIG. 29.—A, curves showing the temperature of peaches in a refrigerator car in transit. Curve *t*, fourth and top layer at the center of the car; *b*, bottom layer, center of the car; t_1, fourth and top layer next to the bunker; b_1, bottom layer next to the bunker. B, development of Monilia (needle inoculations) on Elberta peaches under the temperature conditions shown in A. The position of the heavy dots at the right of the figure shows the actual size of the rots at the destination, and the lines connecting with these trace the growth movement backward according to the values of Figure 2. The broken-line curves show the estimated incubation and growth based on the values of Figures 2 and 4. Curves *et* and *at* give the results under the conditions of curve A, *t*; et_1 and at_1 under the conditions of curve A, t_1; and *eb* and *ab* under the conditions of curve A, *b*. There was no growth under the conditions of curve A, b_1. All inoculations were made two hours before loading

the flesh of the peach in quantity would barely have time to produce a visible rot during the actual transit period, and with cooling such as is shown in Figures 22, 24, and 29 they would not have time to do so; and they also mean that spores lodged on the skin of a peach, even at a puncture, could rarely, if ever, produce a rot during such transit periods as are described in Figures 20 to 30, inclusive. In contrast to this, the ratings for the top of the car would give two and

six-tenths times as many work units as are required for Monilia needle inoculations to produce visible rots and one and one-third times as many as the average required for the production of rots by dusting spores on punctured peaches.

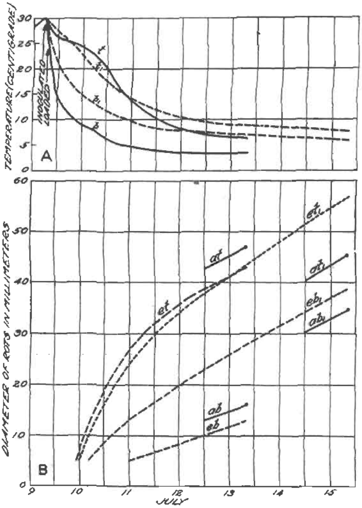

FIG. 30.—A, curves showing the temperatures of peaches in two refrigerator cars in transit and also the temperatures at loading. Curve *t*, fourth and top layer of crates in the fifth stack from the bunker; *b*, bottom layer of crates in the same stack of the same car; t_1, third and top layer of baskets two stacks from the door of the second car; b_1, bottom layer of baskets of the second car in front of the door; t_1, like previous test lots, was in the center of the car crosswise, but b_1 was in the outside row. B, development of Monilia (needle inoculations) on Hiley peaches under the temperature conditions shown in A. The position of the heavy dots at the right of the figure shows the actual size of the rots at the destination and the lines connecting with these dots trace the growth movement backward according to the values of Figure 2. The broken-line curves show the estimated incubation and growth based on the values of Figures 2 and 4. All inoculations were made one hour before loading: Curves *et* and *at* give the results under the conditions of curve A, *t*; *eb* and *ab*, under the conditions of curve A, *b*; et_1 and at_1, under the conditions of curve A, t_1; eb_1 and ab_1, under the conditions of curve A, b_1

With Rhizopus the contrasts between the top and the bottom of the car would be even greater than with Monilia, but not usually so evident in practical results, since in many cases the temperature of the top as well as the bottom of the car was too low for the production of rots during the transit period, even from needle inoculations.

The temperature contrasts between the top and the bottom of the car are composed of two factors, one the temperature level during the last days of the shipment and the other the length of time required to reach this level. The average temperature for the top of the car at the destination was about 4.5° C. higher than that for the bottom, but there was a period of 24 to 36 hours after the cars were closed when the top was 7° to 14° warmer than the bottom. According to the temperature values of Figure 4, nearly 60 per cent of the damage done by Monilia during the given transit periods in either the top or the bottom of the car was accomplished during the first 36 hours after the car was closed. Also, 60 per cent or more of the gain that the rots in the top of the car made over those in the bottom during the transit periods was accomplished during the first 36 hours, and with the shorter and more typical shipments the gain in the top of the car over the bottom was nearly twice as great during the first 36 hours as during the remainder of the period of shipment. The transit period could have been continued one to five days longer, or an average of about 41 hours longer, at the temperatures at which the shipments finished, before the damage resulting from the latter part of the shipment (about 103 hours) would have equaled that produced during the first 36 hours.

In the actual shipping tests, the inoculations were usually made several hours before the fruit was loaded, thus making the final results the combined effect of the car-temperature conditions and the delay in loading, and as previously pointed out (p. 536), the additive effect of the delay was sometimes more significant in the bottom of the car than in the top. In spite of this fact the actual results seem to fully support the differences outlined above.

A study of the growth curves in Figures 20 to 30, inclusive, shows that with the Monilia needle inoculations the actual work units accumulated in the top of the car averaged about 2.5 times as great as those for the bottom of the car, which is in agreement with the estimated values reported above. It should also be noted (based on growth curves continued backward) that, with inoculations made 1 to 6 hours before loading, the rots in the top of the car appeared 17 to 65 hours earlier than those in the bottom of the car and averaged about 42.5 hours earlier.

With Rhizopus needle inoculations made 1 to 6 hours before loading (Table 2), practically no rots started in the bottom of the car, whereas with the exception of the shipments of Figures 28 and 29 a good growth was made in the top of the car.

With Monilia dusting inoculations the contrast in the size of the rots in the top of the car and in the bottom was not so great as with the needle inoculations, but the contrast in the number of rots was especially significant. With the peaches that were punctured and dusted 1 to 6 hours before loading, there was an average of about fifty times as many rots in the top of the car as in the bottom at the destination of the shipment, and with peaches punctured and dusted 14 to 21 hours before loading there was an average of about 4 times as many rots in the top as in the bottom.

Peaches dusted with Monilia spores without puncturing developed about twenty times as many rots in the top of the car as in the bottom.

The regular commercial pack of fruit (Tables 3 and 4) averaged about four times as many rots in the top of the car as in the bottom.

The greater difference between the top and the bottom of the car in the case of peaches punctured and dusted 1 to 6 hours before loading than with those similarly treated 14 to 21 hours before loading would naturally be expected, owing to the fact that the delay gave the rots a chance to start before loading, and they were then able to continue to develop at the bottom as well as at the top of the car. The greater contrast with the dusted peaches than with the commercial pack probably has a similar explanation and is to be attributed to the fact that with the dusted peaches any infections already established are greatly outnumbered by the potential infections, whose development is determined by the temperatures of the transit period.

In commercial shipments the writers have frequently observed that while there are many more rots in the top of the car than in the bottom, there may be no corresponding contrast in the size of the rots. This is apparently due to the fact that the rots in the bottom of the car are confined largely to those that have made some start before loading, whereas many of those in the top of the car have developed during the transit period.

The various differences reported above are between the top and bottom carriers midway between the bunker and the door and do not show the extreme contrasts of the car as found between the bottom layers at the bunker and the top layers at the door. They are sufficient, however, to show the extreme variations in rot production that are often found in the same car.

SUMMARY

A study has been made of the relation of temperature, spraying, and type of infection to the transportation rots of peaches, and an attempt is made to equate the different temperature and infection values.

On the basis of temperature values developed and with the temperature conditions known, it has been found possible to picture the approximate course of development of the rots.

Monilia and Rhizopus rots increased in diameter at an approximately uniform rate in the different stages of development, the diameter of the rots thus becoming a fairly accurate basis for temperature comparisons. For the purposes of the present studies neither the area, nor the volume of the rot, nor the weight of the rotten tissue could be used as a basis for temperature equivalents.

With both Monilia and Rhizopus, low temperatures had a relatively greater inhibiting action upon development during the incubation period than during the later growth. With both incubation and growth a 5° C. change in temperature had a greater effect at the lower temperatures than at the higher ones (experiments ranging from 0° to 30°).

With inoculations made by dusting spores over punctured peaches, the incubation period for Monilia was about twice as long as with needle inoculations, and with spores dusted over apparently sound peaches the incubation period was still further prolonged. A similar contrast was found with Rhizopus, except that the fungus was apparently unable to penetrate the sound skin of market-ripe peaches.

The rots from Monilia dust inoculations continued to appear over a long period of time, whereas those from needle inoculations started off practically together. With Rhizopus there was considerable spread of the infection period in the case of both needle and dust inoculations.

When once established, the Monilia and Rhizopus rots that had resulted from dust inoculations enlarged as rapidly as those from needle inoculations.

Peaches that were punctured and then dusted with Monilia spores developed about four and five-tenths times as many rots as those that were dusted without puncturing and one hundred and sixty times as many as untreated peaches.

Apparently sound unsprayed peaches developed about four times as many rots in transit as similar sprayed peaches.

With dust inoculations and with fruit that had not been inoculated the contrast between the top and the bottom of the car and between delayed and immediate cooling was in the number of rots rather than in their average size. This was apparently due to a delay and spread in the infection period resulting from the lower temperatures and the more rapid cooling.

In the production of Monilia rots the temperatures of the top of the car had about two and five-tenth times the value of those in the bottom of the car, and about 60 per cent of this contrast and 60 per cent of the growth value in both the top and the bottom of the car were developed during the first 36 hours after the car was closed.

Monilia needle-inoculation rots appeared 17 to 65 hours earlier in the top of the car than in the bottom and averaged about 42.5 hours earlier. Peaches that were punctured and then dusted with Monilia spores 1 to 6 hours before loading, developed about fifty times as many rots in the top as in the bottom of the car, and peaches similarly treated 14 to 21 hours before loading developed about four times as many rots in the top as in the bottom of the car.

DEVELOPMENT OF THE BACTERIA CAUSING WILT IN THE ALFALFA PLANT AS INFLUENCED BY GROWTH AND WINTER INJURY[1]

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INTRODUCTION

Bacterial wilt of alfalfa has been closely linked with winter injury of that plant in various ways from its earliest history to the present time. Before bacterial wilt was recognized as a disease caused by a specific organism (*Aplanobacter insidiosum* L. McC.), its symptoms were often ascribed to injury incurred by the plant during an unfavorable winter. After the bacterial origin of wilt was recognized, some of the most destructive epidemics of the disease were observed to follow the partial winterkilling of fields and the severe injury of the surviving plants in which wilt later appeared. This sequence suggested that winter injury made plants susceptible to wilt, though the manner in which this was brought about was not obvious from observation in the field. During the early part of the study of the bacterial disease reported by McCulloch and the writer in a previous paper,[3] no relationship between it and winter injury was discovered.

Later, however, the method found to stain bacteria in the host tissue proved equally satisfactory for the study of the pathological histology of winter injury. Moreover, lesions resulting from freezing often so strikingly resembled those caused by bacteria that it became necessary to study the pathological histology of winter injury before that of wilt could be distinguished with certainty in all cases. This study of winter injury of alfalfa has been published in the second paper[4] in this series. In the course of the comparative study of the two diseases evidence was obtained which indicated that the action of frost upon the tissues of diseased plants served to release the bacteria for distribution in the production of epidemics and also furnished wounds through which they enter and infect plants. Thus the connection between bacterial wilt and winter injury appears to be, locally at least, more important than accidental association or superficial resemblance. It remains, therefore, in this, the third paper in the series, to describe the development of the bacteria in the host plant and the pathological conditions resulting therefrom, and to present evidence that winter injury may provide favorable conditions for epidemics of the disease in infested fields. A brief article on bacterial wilt and winter injury has already been published.[5]

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[1] Received for publication July 19, 1928; issued December, 1928. Cooperative investigations between the Office of Vegetable and Forage Diseases, Bureau of Plant Industry, U. S. Department of Agriculture and the Experiment Station of the University of Wisconsin.

[2] The writer is indebted to many persons for aid in locating and collecting material used in the preparation of this paper, and for suggestions of many kinds. J. L. Weimer, stationed at Manhattan, Kans., has spent much time assisting the writer in collecting material and in interpreting field conditions in Kansas. The drawing presented as Figure 1 was prepared by Dr. Vladimir Skoric.

[3] Jones, F. R., and McCulloch, L. A BACTERIAL WILT AND ROOT ROT OF ALFALFA CAUSED BY APLANOBACTER INSIDIOSUM L. M'C. Jour. Agr. Research 33: 493–521, illus. 1926.

[4] Jones, F. R. WINTER INJURY OF ALFALFA. Jour. Agr. Research 37: 189–212, illus. 1928.

[5] Jones, F. R., and Weimer, J. L. BACTERIAL WILT AND WINTER INJURY OF ALFALFA. U. S. Dept. Agr. Circ. 39, 8 p., illus. 1928.

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METHODS OF STAINING AND COLLECTING MATERIAL

The method of staining that made possible clear distinction between pathological conditions caused by bacterial wilt and winter injury in alfalfa plants was based upon the fact that the bacteria are strongly Gram-positive. The bacteria in the tissue were stained by Gram's stain, usually prepared by Sterling's modified method [6] and the tissue elements were differentiated by staining with safranin and orange G without interfering with the brilliant-blue stain in the bacteria. The Gram's stain served also to stain in a characteristic manner a deposit of material that follows winter injury in comparatively young tissue; and thus, even when the bacteria occur in the same sections with injury, the two pathological conditions are easily distinguished. Details of the staining method have been published previously [7] and need not be repeated here.

After this method of staining had been developed and tested in January, 1926, it was at once apparent that it was more serviceable as a means of determining the presence of the pathogenic bacteria in plants in which they were few in number or mixed with other bacteria than the usual method of identifying them from agar plates prepared from the material. From this time onward, whenever doubtful cases of bacterial wilt were encountered in fields, portions of the suspected plant were fixed in formal-acetic alcohol for future cytological examination. In case it was not convenient to fix the material in the field, the dried roots were sometimes soaked or boiled in water later and fixed and sectioned in the usual manner with satisfactory results in so far as the staining of the bacteria was concerned. Thus it was possible to examine the relation of the bacteria to the host tissues in dried roots collected in previous years.

During the spring of 1926, when the most extensive collection of wilt-infected plants began, a great deal of winter injury was found in both Wisconsin and Kansas, where the larger number of collections was made. At this time the necessity for the comparative study of the pathological histology of winter injury and of wilt became apparent, and collections of portions of plants appearing to show one or both of the diseases were begun for the purpose of checking field observations with trustworthy cytological evidence. Collections were continued during 1927. Usually the upper portion of the root close below the crown was taken in the earlier collections, but later, when the necessity for learning more of the development of the bacteria in the crown was recognized, crowns and stems were collected also. At the time of writing this paper about 200 collections of plants showing wilt, winter injury, or both have been assembled, chiefly from the central Mississippi Valley, and more or less completely examined.

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[6] ZINSSER, H., and RUSSELL, F. F. A TEXTBOOK OF BACTERIOLOGY. Ed. 5, pp. 120-123. New York and London, D. Appleton and Company. 1922.

[7] JONES, F. R. Op. cit.

RELATION OF VISIBLE DISCOLORATION OF WOOD OF ROOT TO LOCATION OF BACTERIA

In the previous paper[3] the most characteristic symptom of the bacterial disease found at all times in the root was described as a yellow discoloration of the outer part of the wood that increased in extent as the disease progressed and that indicated, in so far as could be determined by the isolation of the bacteria on agar plates, the tissue actually invaded by the bacteria. The method of staining the bacteria in the tissue noted here has disclosed the relation of the visible discoloration to the actual location of the bacteria and has shown that the bacteria are not usually as extensively distributed in the wood as the discoloration has suggested. First of all, the discoloration is not due exclusively to the yellow gumlike material previously described in the invaded vessels. Wood may be discolored even before the gum has appeared. This is best seen in newly infected plants. In one collection of recently infected plants showing stripes of pale-yellow color beneath the bark of the upper part of the root, the bacteria were found only in two or three vessels at the centers of the narrower stripes. In such cases the color had diffused to a considerable distance from the bacteria. The solubility of this coloring material was indicated by the fact that the color disappeared after the exposed wood had been soaked in water. This soluble stain appears to be present in varying quantities in the wood of the root, and it may diffuse long distances in badly diseased plants toward the center of the root, unaccompanied by any migration of the bacteria in that direction. This stain is not conspicuously present in stems.

Just as the soluble stain was found to diffuse in the wood far beyond the bacteria, so the yellow gumlike material of undetermined chemical character was found in vessels beyond those entered by the bacteria. The extent of gum formation varies with the location and abundance of bacteria. Usually vessels of autumn wood invaded by bacteria are completely filled with them, and gum is formed slowly and may not extend far beyond the invaded vessels. In summer wood, bacteria may not be abundant in the invaded vessels, forming only thin layers along the sides and between the inner thickenings. Here gum formation may appear in vessels contiguous to those invaded even before it is discernible in invaded ones, and it may extend to all contiguous vessels in the group of which the invaded vessel forms a part, or even to vessels separated by a few small parenchymatous cells, but usually not to those separated by fibers. Thus, one invaded vessel may induce gum formation in five or six others, and a few bacteria well distributed in the vascular system may cause a relatively enormous obstruction of vessels.

The origin and manner of formation of the gum are not easily discovered in sections. It appears to be thin and flocculent in character at first and becomes more firm with age, until at length it is hard and resinous and shrinks away from the walls of well-filled vessels. Sometimes it does not fill the lumen of the vessel but leaves an open

[3] Jones, F. R. and McCulloch, L. Op. cit.

passage in the center. When it fills vessels invaded by bacteria it does not usually engulf the bacteria but crowds them into vacuolelike pockets. (Fig. 1.) From such observation of the behavior of the gumlike material as has been made thus far, it appears to be a product of the alfalfa plant and not of the bacteria.

The invasion of the phloem or of the young stems by the bacteria is not accompanied by the conspicuous and characteristic color changes that accompany invasion of the wood. For this reason the extent and importance of phloem invasion in causing the death of plants was not recognized in the earlier study of this disease. The invaded phloem may be water-soaked in appearance and in time may take on a yellow color, caused apparently by the development of the soluble yellow stain, which usually diffuses so far from the bacteria that it is of little aid in determining precisely their location. The stain has not been seen in young stems. The extent of bacterial invasion outside the wood of the root can usually be determined only by the microscopic examination of stained sections of tissue.

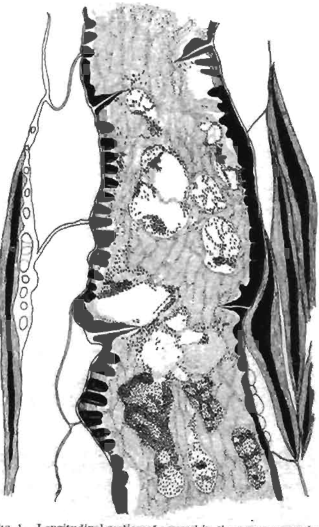

FIG. 1.—Longitudinal section of a vessel in the summer wood of an alfalfa plant infected with wilt. This material appears to have been fixed after gum formation was complete but before the gum had begun to shrink away from the vessel wall. The bacteria are found first characteristically along the vessel wall. The gum, formed presumably by the surrounding cells, has pushed nearly all of the bacteria away from the wall. × about 800

PROGRESS OF BACTERIA IN HOST PLANT FROM INFECTION TO DEATH OF PLANT

INVASION OF WOUNDS AND ENTRY OF VASCULAR SYSTEM

The infection of the plant by the bacteria producing wilt is, so far as is known, wholly through wounds. The wounds may be of such character that entry may be made in two very different ways. The bacteria may be introduced directly into the vascular system through the cut ends of stems, or they may enter between cells of parenchymatous tissue through which they force their way to the vessels. The first method of infection, while effective in artificial inoculations, appears to be unusual in the field. In nature it appears that the bacteria usually reach the vessels through parenchymatous tissue. Wounds in the root, through which the

bacteria may gain entrance, expose phloem parenchyma, and, similarly, wounds in the crown stems, at least those resulting from winter injury, also expose phloem parenchyma after the sloughing off of the cortex. Inasmuch as the behavior of the bacteria in the tissues exposed by wounds, whether in roots or stems, is essentially the same, invasion through wounds in roots will be traced for present illustrative purposes.

Wounds in the phloem when made artificially may in old roots penetrate only tissue several years of age that is greatly crushed from internal pressure. Such wounds if extensive do not usually remain superficial. The internal pressure that crushed the outer tissue, no longer opposed by the encircling bark, often leads to a cracking of the phloem along a phloem ray nearly down to the cambium, or at least

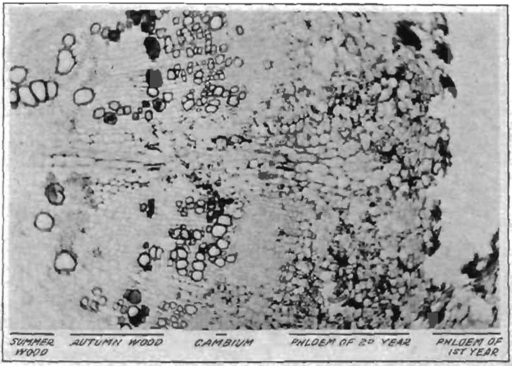

FIG. 2.—Photomicrograph of a portion of a stained cross section of a 2-year-old alfalfa root through a wound from which the bacteria producing wilt entered the vessels of the underlying vascular bundles. The bacteria are represented by the black masses in the vessels and between the parenchymatous cells. The black lines passing from the wound in the phloem along the ray cells through the interfascicular cambium into the xylem represent the course taken by the bacteria in entering the vascular bundles. In the central xylem ray the bacteria have passed a short distance into the zone of summer wood. At the right the bacteria have caused collapse of immature cells near the youngest vessels invaded. Some of the finer lines of bacteria between cells and scattered bacteria clinging to the inner walls of vessels that are not largely plugged are not shown in the photograph. The root used in this artificial inoculation was transplanted from the field inoculated November 3, 1925, and fixed January 26, 1926. × 76

permits the enlargement of ray cells and the opening of large intercellular spaces as the tissue is pulled apart by tangential expansion. Cork cambium formed to protect the wound may be broken again and again because of insufficient elasticity in this direction. Thus wounds even when superficial often expose sooner or later the deeper active layers of phloem.

When wounds are made in comparatively young phloem, as shown in Fig. 2, and the parasitic bacteria are brought in contact with such wounds, infection takes place readily. The precise manner in which the bacteria first make their way into the intercellular spaces of the parenchymatous tissue is a matter of conjecture. It is not improbable that they are carried into the intercellular spaces by

capillary water, as in the following experiment: When the outer phloem of roots of growing plants is sliced off with a sharp knife under a bacterial suspension and the cut tissue is immediately fixed and stained, the bacteria are found in the larger intercellular spaces of the phloem to a depth of two or three cells from the surface. Apparently this tissue absorbs water strongly, and the bacteria are carried into the larger intercellular spaces with the water. The entrance of the bacteria into the plant tissue through natural wounds may take place in the same manner.

From the place of entry the bacteria spread through the larger intercellular spaces in all directions. Progress is most rapid along the phloem rays to the interfascicular cambium, which, unlike the fascicular cambium, is almost always composed of comparatively large cells with intercellular spaces. It may be noted here, however, that the bacteria are not limited to intercellular spaces. In certain young but fully expanded tissue the bacteria are often found surrounding cells in a thin layer scarcely more than one bacterial cell in thickness, as though they had grown through the middle lamella when it was in a plastic condition. Solution of the lamella is not evident. The interfascicular cambium does not usually appear to be damaged by the passage of the bacteria. In some cases it seems to have resumed growth after the bacteria have passed through it and to have separated the invaded parenchyma of the phloem from that in the wood with uninvaded tissue.

After passing the interfascicular cambium, the bacteria continue along the wood ray through about half the last annual increment laid down at the time the bacteria pass the cambium, unless, as sometimes happens in the spring, the deeper ray tissue has been previously split apart by winter freezing. Even when passage along rays is thus facilitated by the physical separation of cells, the bacteria do not usually develop abundantly in the older wood.

As soon as the bacteria have passed the cambium they begin to spread tangentially along the middle lamella between the parenchymatous cells of the vascular bundles until they reach the vessels. Occasionally they advance through immature cells close inside the fascicular cambium, causing the disintegration of these cells and the formation of bacterial pockets contiguous to young vessels. (Fig. 2.) The outer wall of the vessel appears to offer no resistance to the entry of the bacteria.

The bacteria do not continue to spread indefinitely among the cells of either phloem or xylem from the point of infection. Their spread seems to be slowed up largely by the growth from the cambium and the maturing of the tissue in which they are located during their slow advance. The relation of growth of the bacteria to the maturity of the tissue will be discussed in a later section.

DISTRIBUTION OF THE BACTERIA THROUGH THE VASCULAR SYSTEM

Soon after the bacteria have entered the vessels of two or three of the bundles in the manner shown in Figure 2 they are found in corresponding vessels in all the bundles around the circumference of the root and far up the stem as well as down the taproot. This comparatively rapid spread of the bacteria in the plant is one of the most important phases in the development of the disease, and therefore

the manner in which it is brought about deserves special attention. Unfortunately, it is very difficult to follow it by direct observation.

There are three possible routes by which the bacteria may pass from vessel to vessel: First, by open passages between vessels; second, by penetration of the thin middle lamellalike walls separating contiguous vessels; and, third, by passing through the middle lamellae of walls of parenchymatous cells, separating vessels precisely as in the first invasion of vessels described.

Distribution Through Open Channels

The open communications between vessels through which bacteria may pass without actually penetrating cell walls will first be examined. In a vertical direction in root and stem long open communicating vessels are easily demonstrated. If taproots of vigorous plants several years of age are cut with a razor under india ink in suitable dilution, the ink will often pass up or down from the cut 6 to 7 cm. in half an hour. In this way open communication through vessels is easily demonstrated from stems through crowns to the root. The extreme length to which these open passages may reach has not been determined, but at least they are sufficiently long to account for much of the vertical distribution of the bacteria in the plant through open channels alone.

This method of demonstrating the length of open vessels in the alfalfa plant reveals an added fact that is of interest here as well as in a later connection. The vessels through which the living plant will draw the ink to considerable distances when a cut is made under the ink suspension are located exclusively in the outer part of the root in the precise region where the bacteria distribute themselves whether they enter the vessels through wounds or are introduced like the ink through cut vessels. This experiment has been repeated during the summer, and, though the results show some variation, the conducting vessels demonstrated in this way are usually within one-half of a year's growth from the cambium.[9] The small vessels of autumn wood tend to retain conductivity slightly longer than the larger ones of the midsummer wood, and thus late in the summer when the early summer wood fails to draw the ink the wood of the previous autumn may still draw it a long distance. Since no obstructions have been found in the vessels that do not draw the ink, the assumptions made by MacDougal et al.[10] as a result of similar experiments with trees seem valid here; namely, that the vessels that fail to conduct the ink for long distances are in fact filled with air, not with water. Thus the fact that the inner vessels of infected plants never become filled with bacteria may be due not only to the inability of the bacteria to traverse the parenchyma far enough to reach them, but also to the absence of water by which the bacteria may be distributed through them.

Lateral openings in a tangential direction between vessels in sufficient number and of suitable arrangement to insure the open-channel distribution of the bacteria around the entire circumference of the

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[9] When these experiments were repeated in 1928 at Madison, Wis., many vessels in the spring wood of that year were found open and filled with water in the autumn in nearly all plants. Thus, contrary to findings in the previous year, the entire ring of 1928 wood retained its ability to conduct water throughout the growing season. Unfortunately, no inoculations were made at suitable dates to determine whether infection in the autumn could be followed by distribution of the bacteria through all the vessels actually filled with water. It seems unlikely that this would have happened, and therefore the conclusions drawn from the earlier observations are retained until they can be corrected, if necessary, by future work.

[10] MacDougal, D. T., Overton, J. B., and Smith, G. M. THE GAS-WATER SYSTEMS OF CERTAIN WOODY ESTMS. (Abstract) Amer. Jour. Bot. 14: 622-623. 1927.

root are not so easily demonstrated. However, open communication between vessels can be found. The vascular bundles, especially in the upper part of the root, pursue zigzag courses, joining lattice fashion around the wood rays. At the point of junction these rays when seen in cross section are marked by greater width, and in these wide places the converging vessels are often arranged side by side in rows across the bundle. These rows of vessels may fail to complete the contiguous lateral walls, thus leaving an open channel between them formed in precisely the same manner as the openings between the vessel segments. Sometimes three or four vessels converging from the two bundles have a common communicating channel in the same horizontal plane. (Fig. 3.) Whether or not all vessels communicate in this manner has not been determined. Each bundle converges with another bundle in the vascular net at each 2 or 3 mm. of its length. If each vessel has, as seems likely from the previous experiments, an open channel at least 6 cm. long, it passes in the course of its length through at least 20 vascular junctions where connections with other vessels may take place. In diseased plants masses of bacteria have been found connected from one vessel to another through such openings, and it appears that the bacteria are distributed around the circumference of the roots to some degree if not entirely through these open vascular connections.

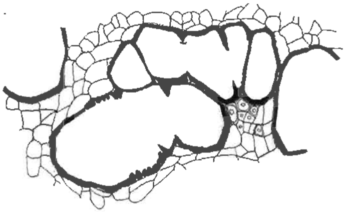

FIG. 3.—Open communications between vessels at the convergence of two vascular bundles of the alfalfa plant. The lateral walls are absent from the vessels precisely as the end walls are absent from the vessel segments. Openings of this kind have not been found between adjacent vessels in single bundles, and they are not often found opening in a radial direction. Vessels opening in this manner are also shown in Figure 2. × about 1,000

Open connection of this character in a radial direction between contiguous vessels has been found in but a single instance. It appears to be so rare that it can not account for the radial spread of the bacteria into new wood produced by the cambium.

DISTRIBUTION BY PASSAGE THROUGH MIDDLE LAMELLAE OF WALLS OF CONTIGUOUS VESSELS

Inasmuch as it has been shown that the bacteria pass readily between parenchymatous cells and also appear at times to pass between vessels, it may seem highly probable that they can pass from one vessel to another contiguous vessel by penetrating the lamellae separating their lumina wherever the open spaces between the inner thickenings are opposed. A clear demonstration of this seemingly possible method of distribution has not yet been made. Certainly the bacteria do not always pass readily from one vessel into contiguous vessels, though it is probable that they do so in young tissue. Again and again in longitudinal sections bacteria are found confined to a single vessel, though it may be in contact with several others. Inasmuch as this method of passage of the bacteria from vessel to vessel has not been demonstrated and does not seem necessary to explain the distribution of the bacteria, it will not be discussed further.

PROGRESSIVE INVASION OF YOUNG VASCULAR TISSUE BY ADVANCING THROUGH PARENCHYMATOUS TISSUE

In the microscopic sections made from the upper part of the taproot of old diseased plants the parasitic bacteria may be found distributed through several annual rings of wood; but only rarely does a section reveal how the bacteria may have passed from the early inner wood to the more recent outer wood. The bacteria appear to invade new wood by advancing through young parenchymatous tissue chiefly young wood ray cells inside the cambium and entering the bundle and the vessels as soon as they have completed growth. The adjustment between host and parasite in this process may be so well made that the bacteria advance through the plant for several years without killing it. The essential features of this invasion may be seen in Figure 2, which shows the invasion of the vascular system by bacteria entering through a wound. In this figure the first vessels entered by the bacteria were undoubtedly the inner vessels showing plugging from the bacterial mass and gum. This probably took place before the outer vessels shown in the photograph were fully differentiated and developed. As these vessels reached mature size, the bacteria, following between the expanding interfascicular cells, were at all times ready to enter the bundle as soon as its maturity permitted, and thus the vessels were invaded in succession. Nearly all of the vessels in this younger wood are actually invaded and along their inner walls have bacteria too few in number to show in the photograph. It will be noted that at the right of the central ray the bacteria have invaded immature fascicular tissue, causing its collapse and the formation of bacterial pockets adjoining the invaded vessels. Bacterial pockets of this kind are not always formed in this situation and the bacteria may continue to develop between the cells close behind the interfascicular cambium for several years, invading successive layers of wood without producing much visible disorganization of the tissue and without inciting the tissue to inclose the invaded area with dividing cells as in a wound. The invasion of new wood does not always continue indefinitely at the place where the bacteria entered, but it may proceed at other places in the crown where bacteria have entered parenchyma from vessels. This will be discussed further in connection with the description of the progress of the bacteria through the parenchymatous issue.

INVASION OF PARENCHYMATOUS TISSUE

Certain characteristics of the invasion of parenchymatous tissue by the parasitic bacteria have been discussed. It has been noted that the bacteria progress more rapidly along the larger intercellular spaces; that they flourish best in comparatively young tissue but do not necessarily enter undifferentiated tissue near the fascicular cambium; and that the bacterial advance is very slow in comparison with that in most bacterial diseases—so slow that growth and maturity in the tissue invaded must usually be taken into consideration in the study of the development of older lesions. In the examination of the relation of the growth of the plant to bacterial spread it is soon apparent that growth made during the summer is relatively highly resistant to the bacteria compared with that made during the autumn. An apparent exception to this generalization may be found in badly diseased plants in which the summer growth

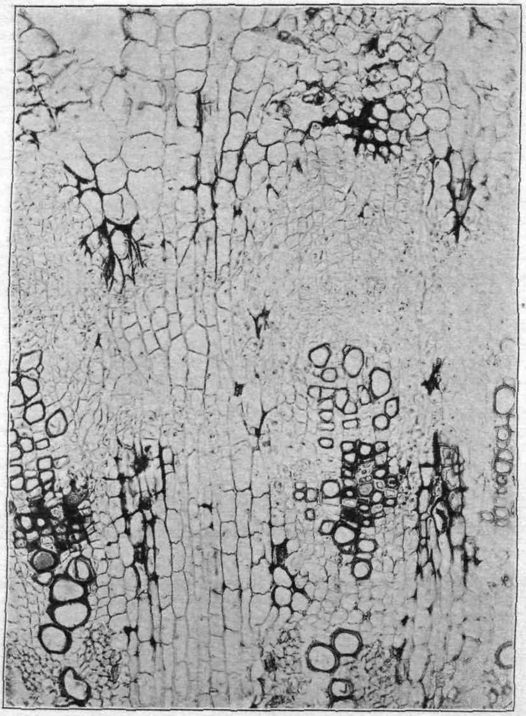

FIG. 4.—Photomicrograph of a stained section of the youngest portion of two vascular bundles in the upper part of the root of a 2-year-old alfalfa plant suffering severe winter injury. The plant was collected at Madison, Wis., May 5, 1927. This root when removed from the ground showed yellow discoloration under the bark very much like that in bacterial wilt, except that it did not extend far below the crown. The section shown here was stained precisely like those stained to reveal the parasitic bacteria. The youngest vessels of large diameter may have been produced in the spring following injury. In some of the small vessels of the autumn growth a gum is found similar in appearance to that in bacterial wilt. The deeply staining material between parenchymatous cells is deposited in and between cells that appear to have been separated mechanically by frost action. Thus winter injury of this character not only simulates bacterial wilt in the discoverable symptoms in the field, but the stained microscopic preparations show a striking superficial similarity to those of plants infected with bacterial wilt. Compare with Figures 2 and 5. × about 200

sometimes resembles that normally made in the autumn. On the whole, however, from the examination of the cross sections of roots it is clearly seen that the spread of the bacteria in autumn wood and perhaps in early spring wood is far greater than that in summer wood. If plants are able to grow vigorously in the summer, the advance of the disease is arrested. In other words, the susceptible tisssue located on either side of the cambium may be described as consisting of hollow cylinders of phloem and xylem connected with each other through the interfascicular cambium. The cylinder of susceptible xylem becomes very thin or perhaps disappears in summer, and reaches a maximum thickness in late autumn and early spring. Curiously enough, these cylinders of tissue susceptible to bacterial invasion are precesely the region in the plant that responds most vigorously to winter injury with the deposit of the material in the cell walls or between the cells. This material stains in a characteristic manner with Gram's stain, as described previously. Sections of roots in which the parasitic bacteria are stained often present a striking similarity in appearance under low magnification to injured but bacterium-free roots stained by the same method. This similarity is shown by a comparison of Figure 4, which is a photomicrograph of a stained section of a root injured by winter freezing, with Figures 2 and 5, which are photomicrographs of similar tissue invaded by the bacteria and stained by the same method.

In the preceding paragraphs consideration has been given only to the development of the bacteria in the parenchymatous tissue of the secondary growth in roots. Secondary growth in the crown is for the most part similar in character to that in roots, and bacterial invasion proceeds in essentially the same manner, though oftentimes apparently more rapidly. The relatively large amount of tissue favorable for invasion and the less compact cellular arrangement in the crown seem to furnish the bacteria favorable conditions for growth. At the bases of the numerous stems and also of the lateral roots the phloem parenchyma is often abundant and seems to be especially favorable for the formation of bacterial pockets. The broken bases of stems are sometimes partly covered with a loosely arranged callus through which the bacteria may become widely distributed. This extensive bacterial invasion of secondary growth in the crown is not often conspicuous because of resulting discoloration or death of tissue. It is important because these bacteria are in a peculiarly favorable situation for invading vascular tissue at an active region of growth where efficient distribution of bacteria through new growth in the taproot below and the primary structure of stems above is assured.

The bacteria may pass up into the innermost vessels of the primary structure of stems at a very early stage in their development and pass from the vessels to parenchymatous tissue even more readily than in roots. Inasmuch as the interfascicular tissue of the outer part of the primary structure and all of the secondary growth is heavily lignified, the bacteria are prevented from passing outwardly as in roots, though they enter the pith readily; and, therefore, in the true stem the bacteria seem to do comparatively little harm, though they may pass almost to its very top. The extent to which stems may be invaded is described more conveniently in a later section.

The basal portions of stems, especially of those produced from the lower part of the crown in autumn, have the morphological character-

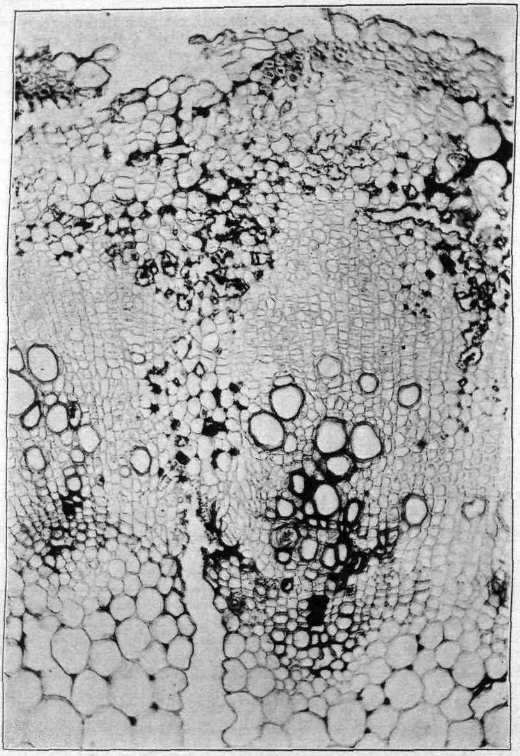

FIG. 5.—Photomicrograph of a stained cross section of a small crown stem or stolon of Semipalatinsk alfalfa, showing two vascular bundles invaded by the wilt-producing bacteria. Infection of the stolon had evidently taken place in the autumn from bacteria advancing through vessels from the invaded root. The inner vessels are plugged and a considerable distribution through parenchymatous tissue had taken place. Freezing injury during the winter destroyed the tissue exterior to the bundle caps shown at the margin of the section, and caused some separation of cells in the pith and the phloem. Some of the large vessels of the wood are probably of spring growth. Although growth at the cambium has begun, note that very little protective cork has formed at the exterior of the wound. At various places in the sections of this stem masses of bacteria were manifestly being released by the disintegration of the outer injured tissue. × about 200

istics of the crown from which they arose. Here the interfascicular tissue is not lignified either in the primary structure or in secondary growth. Therefore, in the crown structure the bacteria pass from the vessels both inwardly through the pith and outwardly as far as

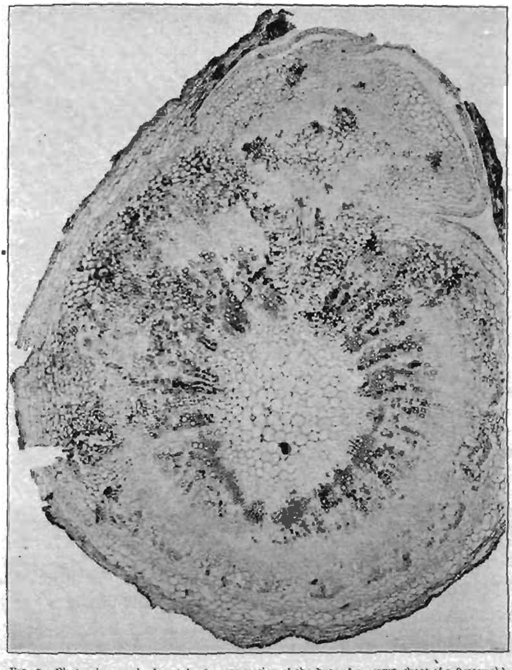

FIG. 6.—Photomicrograph of a stained cross section of the base of a crown shoot of a 2-year-old alfalfa plant badly diseased with bacterial wilt. The plant was collected at Monroe, Wis., November 29, 1927. The extent of bacterial invasion of the tissues at this date is shown by the black masses of bacteria extending all the way from the pith to the cambium on one side of the stem. In general appearance this shoot resembled those shown in Figure 9. Already the primary stem tissue outside the endodermis is cracking open from the diseased condition beneath. The fate of stems like this after heavy freezing is shown in Figure 10. × 40

the endodermis. The extent to which stem bases of primary crown structure may be invaded in the autumn shoots is shown in Figure 6. Thus the primary structure of the stem bases or crown appears to be a very favorable place in the plant for abundant bacterial develop-

ment. The significance of this fact will appear in a subsequent discussion of the relation of winter injury to the spread of bacterial wilt.

INVASION OF STEMS IN SEED-BEARING PLANTS

The migration of bacteria up the stems of seed-bearing plants has been examined with a view to determining whether or not the bacteria actually enter the seed. In the autumn of 1925 infected plants bearing seed were collected in Kansas and Idaho, and in 1926 further collections were made in Kansas In both years comparatively few infected plants were found producing seed. With the aid of a microscope many of these were examined in the field for the presence of bacteria in the stems. Comparatively few of the stems showed evidence of the presence of bacteria very far above the crown. Those showing considerable stem invasion were dried and brought to the laboratory for examination. The distance to which the bacteria had advanced in considerable number was determined by pouring agar plates from dilutions or macerated stem fragments from successively higher nodes. When a node was reached at which the bacteria appeared to be very few, fragments at still higher levels were embedded, sectioned, and stained by Gram's method. In this way the bacteria were traced in one plant collected in 1925 to the base of the pedicel bearing a seed pod, and in 1926 the presence of bacteria was demonstrated in the base of a seed pod by staining, but they could not be found near the hilum of the proximal seed in the pod. At the highest point at which the bacteria were found they were not only in vessels but also between parenchymatous cells. In no case were the bacteria found abundantly in the upper parts of the seed plants. Thus seed invasion was not demonstrated, and from the examination of material in these years it seems unlikely that it takes place and certainly that it does not take place abundantly.

EXTENT OF DEVELOPMENT OF BACTERIA IN PLANT ACCOMPANYING APPEARANCE OF SYMPTOMS OF DISEASE IN FOLIAGE AND DEATH OF PLANT

In the preceding description of the invasion of the bacteria through various parts of the plant no attempt has been made to correlate this invasion with the development of the characteristic symptoms that appear in the foliage before the plant dies or to state precisely how the death of the plant is brought about. Inasmuch as most of the plants examined have been sectioned only at one selected portion of the axis for examination of the development of pathological conditions, it is not possible to state with precision the relation of the bacteria to the plant as a whole as the disease progresses. However, it appears that the mechanical obstruction of vessels in the actively conducting wood, together with the cessation of the formation of a new vascular system, accounts largely if not wholly for the dwarfing and death of infected plants. The obstruction appears to be, as a rule, most important in the upper part of the taproot and crown. It is brought about not so much by the bacteria, though they may fill vessels of autumn wood very full, indeed, as by the gumlike material deposited by the plant in vessels in the region of invasion.

In artificial inoculations bacteria may be distributed so widely in the vascular system that they may multiply, plug vessels, and kill the plant before gum is formed. Figure 7 illustrates such a case. But natural infection has not provided instances of this kind.

In old plants growing under conditions where little wood is added each year, the death of the plant may be brought about by the plugging of a comparatively narrow layer of wood. When vascular plugging is sufficiently abundant to retard normal increase in diameter of the root in summer, foliage may begin to wilt on hot days. Up to this time parenchymatous tissue is not usually invaded extensively. When root growth is retarded the new wood contains fewer fibers, and the vessels are usually smaller in diameter and are invaded very

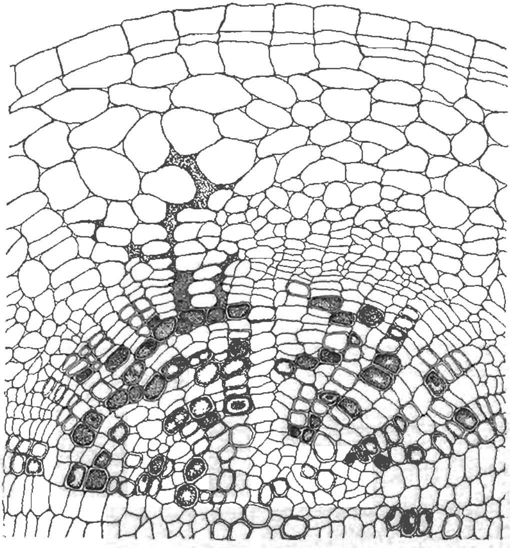

FIG. 7.—Portion of a cross section of the upper part of the root of a small alfalfa seedling inoculated with *Aplanobacter insidiosum* by cutting the stem with a razor dipped in bacterial suspension. The seedling was dying when taken for examination. Note that nearly all of the vessels are filled with bacteria, that no gum formation has yet taken place, and that in one place the bacteria have passed from the vessels into the parenchyma and through the cambium into the phloem. This is the characteristic distribution of the bacteria in the root of small infected seedlings. × 540

romptly. The vascular rays are invaded more extensively at this tage, bacterial pockets may be formed in the slow-maturing young ells near the cambium, and the bacteria pass out through the interascicular cambium into the phloem. During this stage in developnent of the disease the shoots produced after cutting are short, with nall leaves characteristic of the disease. When the bacteria have ntered the phloem around a large part of the circumference of the ot near the crown the plant does not

favorable external conditions. Before the plant dies the disease may appear to have become parenchymatous in character. Usually the bacteria do not advance into the phloem uniformly around the entire circumference of the root, and the plant may die when they have begun to destroy parenchymatous tissue around less than half of the cambial strands.

LONGEVITY OF WILT-INFECTED PLANTS

EVIDENCE OF LONGEVITY FROM CYTOLOGICAL STUDY

In the earlier part of this paper it has been shown that the first vessels invaded by the bacteria after infection are the outermost ones close beneath the cambium, and that vascular invasion never proceeds inward from this position. Thus it is obvious that however long the plant may live subsequently, these inner invaded vessels will remain as guideposts, marking the time of infection as long as the annual rings of growth can be distinguished. In most plants the summer and autumn layers of each annual ring are readily recognized. Moreover, the parasitic bacteria appear to remain Gram-positive after years of imprisonment in the tissue. Thus from stained sections of roots of infected plants the year in which infection took place can be determined. If the infected plant was growing with moderate vigor, and adding considerable wood each year, it is also possible to determine, approximately, at what time in the year the bacteria entered the vessels. This fact became apparent to the writer during the comparative examination of sections of plants inoculated in the field at various times during two summers at Madison, Wis. Inasmuch as it is a matter of considerable importance to determine at what time plants are infected in the field, the sections of roots of plants inoculated artificially were used as standards of comparison by which the time of infection of diseased plants collected in many fields was estimated. Since the annual growth of most of the field plants was small, it was not often possible to estimate the date of infection closely. The task was simplified, however, by the discovery that in a large majority of the plants the innermost invaded vessels were in approximately that portion of the annual ring where spring infection was indicated. Evidence of infection in autumn was rare and of doubtful authenticity. Therefore in the estimation of the time of infection of diseased plants those having the innermost invaded vessels in autumn wood were usually regarded as infected in the spring, and those having innermost invaded vessels in late spring or summer wood were designated as infected in the summer.

The oldest record of infection with wilt and subsequent recovery thus far found was obtained in the spring of 1926 from a field said to be 17 years old, located near Abilene, Kans. At the time the collection was made only a few plants showing symptoms of disease could be found; but some of the more vigorous, when dug, disclosed discolorations arranged in a circular manner near the center of the large taproots. When these taproots were sectioned and stained, bacteria were found in the discolored areas. Unfortunately, the annual rings of growth in these roots were so narrow and so poorly differentiated that not all of them could be made out with certainty. However, it appeared from the number of bands of crushed phloem that these plants were at least 15 years old. One root was hollow at the center

and had lesions in the cortex, indicating winter injury in the second winter of the plant's growth. The parasitic bacteria were demonstrated in vessels close to the corky layer that surrounded the hollow center, and also in four or five subsequent annual rings, in the last of which they were quite abundant. Beyond this region no evidence of infection was found. In the second root a lesion in the parenchymatous tissue of the wood, which was interpreted as caused by winter injury at the end of the second or third summer's growth, was the innermost point of invasion by the bacteria, which were distributed outwardly through all but the last three annual rings. Thus these two plants appear to contain records of infection by bacterial wilt following winter injury at least 12 years prior to 1926. If this interpretation is accepted, it is clear that the bacterial disease has long been established in this locality, though it has been recognized but recently.

Other instances of recovery and long life of infected plants have not been found. The central wood of the roots of old plants rarely escapes decay as long as 15 years except under conditions of very low rainfall. In fact the plants from Abilene described above were growing in a small tract of sandy soil, and they resembled plants from semiarid districts in having narrow annual rings and little autumn wood.

A large part of the diseased plants from various sources whose roots have been sectioned were showing symptoms of disease when dug and had been infected two or three years previously. Many of them had undoubtedly been infected through injury received in the winter, but in the earlier collections care was taken to select portions of root showing no external injury, and therefore evidence of injury does not appear in many of the sections. For this reason correlations between winter injury and time of infection with wilt can not be satisfactorily made. It is possible, however, in many of the sections to determine approximately the time at which infection took place and the duration of the disease in the plant prior to collection.

The records from 113 plants collected during three years are given in Table 1. From this table it appears that practically three-quarters of the plants collected at random show infection in the spring. In fact, the majority of those indicating infection in the summer are from fields in Kansas, collected after the heavy summer rains of 1927. It may also be inferred that most infected plants live at least one year, and sometimes much longer. This table can not, however, be regarded as an approximate table of life expectancy of infected plants, because of the irregular manner in which the plants were collected.

TABLE 1.—*Time of infection with reference to time of collection of 113 alfalfa plants infected with Aplanobacter insidiosum and collected in 1925–1927*

Year of infection	Number of plants that showed spring infection	Number of plants that showed summer infection
Year collected	25	15
Year previous	38	4
Second year previous	27	3
Third year previous	1	
Total	91	22

LONGEVITY OF PLANTS INOCULATED ARTIFICIALLY

From the preceding discussion it is apparent that the longevity of infected plants may vary considerably in different localities and under different cultural conditions. Experiments have been undertaken to determine the usual length of life of infected plants at Madison, Wis. For the first of these experiments a plot of 3-year-old alfalfa containing strips grown from seeds of the Grimm, Utah, and South Dakota varieties was contributed by F. L. Graber. This was inoculated July 31, 1925, cutting the plants close to the ground with a scythe kept wet with a bacterial suspension. During the rest of that summer only one plant showed symptoms of disease in the foliage. During the following winter, winterkilling damaged the Grimm and nearly destroyed the stands of the other two varieties. Nevertheless the surviving plants grew vigorously. The first cutting of this plot in 1926 was made June 18, and the first symptoms of disease in the surviving plants appeared July 2. By July 15 at least one-half of the surviving plants showed indications of disease, and 165 of these in one corner of the plot were marked with wires for future observation. On September 20, 85 of the marked plants were dead and the majority of the remainder had dwarfed yellow foliage. During the following winter nearly all the plants in the plot were winterkilled and the experiment was discontinued.

This and other experiments designed to test the longevity of infected plants at Madison have been interfered with so badly by winterkilling during the last three years that clear experimental evidence of the usual length of life of infected plants under Wisconsin conditions can not be presented. However, from this experiment and other work now in progress it appears that the usual course of the disease is as follows: Infection takes place chiefly in the spring; few of the infected plants show visible symptoms in the foliage during the following summer, unless winter injury associated with the infection is severe; symptoms begin to appear early in the second summer after infection; and a majority of the infected plants are dead by the end of that summer or are too weak to survive the winter. A small number of the diseased plants live into the third summer after infection, and most of these survivors perish before that summer is past.

WINTER INJURY IN RELATION TO BACTERIAL WILT

WINTER-INJURY LESIONS AS POINTS OF ENTRY FOR BACTERIA CAUSING WILT

The first direct evidence that lesions caused by winter injury serve as foci of infection by the bacteria producing wilt was obtained in the spring of 1927. Among the wilt-infested fields found near Monroe, Wis., in the fall of 1924 was an old stand with a new seeding made that year on lower ground adjoining. The new seeding became infested, and in 1926 a third seeding was made on still lower ground adjacent. The winter of 1926–27 damaged severely both the new seeding and the 3-year-old field. In the latter field many plants were killed and nearly all survivors showed winter injury. Of those remaining in the young stand practically all were likewise damaged in the crown and upper part of the taproot. Heavy showers in the spring of 1927 caused some washing of soil and débris across the two fields.

On June 5, during examination of damaged plants in the youngest field, yellow streaks indicative of wilt infection were found in the wood of many roots. Four of these plants with yellow streaks in the lower part of the root were selected, and the entire upper part of the root and crown was fixed for cytological examination. Sections were made at several selected places in root and crown. In each of these plants winter-injury lesions were found, with abundant bacteria in the surrounding uninjured tissue, the bacterial invasion extending in places all the way to the vessels through which the bacteria had passed both upward and downward, giving rise to the yellow streaks noted in the lower part of the taproot when the plants were collected.

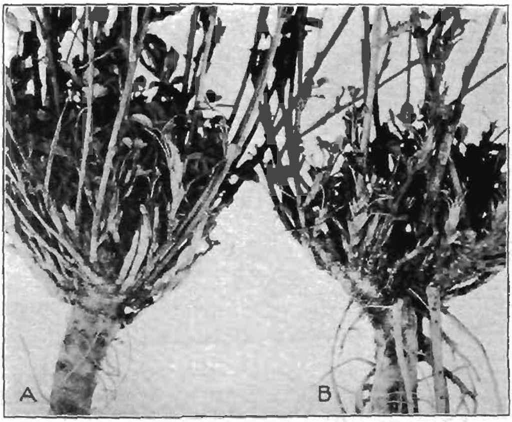

FIG. 8.—Crowns of 4-year-old Grimm alfalfa plants infected with bacterial wilt, found at Monroe, Wis., November 26, 1927, showing the development of young shoots when winter conditions arrived. The taller stems were killed by frost, but the short ones were apparently alive and uninjured at this date. The more severely diseased plant (B) shows small leaves characteristic of the disease. One of the shoots on this crown is shown in Figure 9. × ⅓

The distribution of the bacteria from the winter-injury lesions suggested that the bacteria had entered from them precisely as they had entered wounds in artificial inoculations previously described. The position of the innermost invaded vessels and the comparative absence of gum formation in vessels near those invaded both indicated recent spring infection. In fact, a search of this field as recently as May 5 had failed to detect any diseased plants. The source of the bacteria producing infection was undoubtedly in the older diseased field, in which many plants had been killed and from which the bacteria could easily have been carried in surface water.[11]

[11] In May, 1928, the infection of plants in a field seeded in 1927 was again traced through wounds caused by winter injury. As in the preceding year, the bacteria could be stained in continuous lines between living cells from the exterior of the wound to the vessels.

RELEASE OF BACTERIA FROM DISEASED PLANTS BY WINTER INJURY

In the autumn of 1927 special attention was given to the development of buds and shoots on both healthy and diseased alfalfa plants, preparatory to a study of the action of freezing upon these shoots during the winter and to the search for new infections the following spring. In the course of this work remarkable development of the bacteria in the bases of shoots formed in the autumn was found. The autumn was unusually warm and moist. The average daily temperature in excess of the average in September, October, and November at Madison, Wis., was 2.4°, 3.5°, and 2.2° F., respectively, while rainfall for the three months was 3.4, 1.81, and 0.62 inches, respectively, in excess of normal. Probably the high temperature and rainfall forced the autumn growth more than usual, though in the absence of precise observation in previous years this can not be determined.

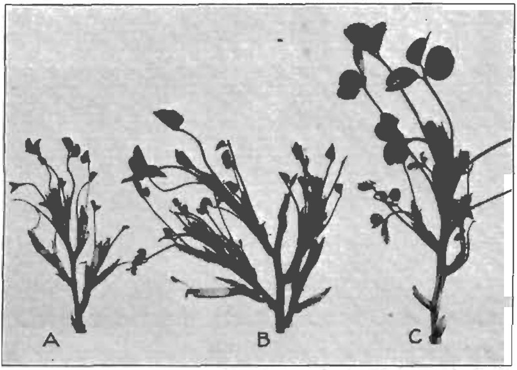

FIG. 9.—Crown shoots of diseased Grimm alfalfa plants (A and B) collected at Monroe, Wis., November 26, 1927, compared with a shoot from a healthy plant (C) in the same field. The central shoot (B) was taken from the plant shown as B in Figure 8. A cross section of the base of the stem of this central shoot was found to be invaded by the parasitic bacteria in a manner very similar to that shown in Figure 6. × 2

The development of shoots from the crowns of 4-year-old diseased Grimm alfalfa plants at Monroe, Wis., on November 27 is shown in Figure 8. These crowns could not be distinguished from those of healthy plants by casual observation. The taller fall stems developed after the last cutting had been killed by frost, but many of the autumn buds had grown out to form a dense green tuft close to the ground. Some of the shoots arising from the bases of these diseased crowns had small leaves characteristic of the disease. One of the shoots from the second plant in Figure 8 is shown in Figure 9. Although clearly showing disease in the foliage, the stem failed to show in a cross section taken at its base more than a very slight water-soaked appear-

ance and a trace of yellow hardly sufficient to distinguish it from a healthy stem; yet a stained cross section of this stem revealed many parasitic bacteria, not only in the vessels, but also in even greater numbers between the parenchymatous cells of phloem and pericycle. The cortex had already broken over the largest bacterial mass, and some of the bacteria may have been released into the soil. After a little experience in searching for these invaded stems at the bases of the crowns of diseased plants they were found in considerable number, though usually only a few were obtained from a single plant. Apparently the autumn stems are similar to autumn wood in the root in possessing a structure through which the bacteria pass readily and in which they may develop in great numbers without immediate destruction of the tissue.

The importance of this abundant development of bacteria in young shoots lies not so much in the damage that can result from the killing of the shoots—though when invaded as badly as that shown in Figure 6 the shoot will not develop far—as in the situation of these bacteria at the surface of the soil in succulent tissue from which they may be easily released and conveyed to other plants. As early as the end of November, before the shoots were injured by frost, the diseased stems were sometimes found cracked to such an extent that a few bacteria must have been released, and it was obvious that winter freezing might serve to release a large part of them. The release of the bacteria by frost action was demonstrated in shoots collected from the same field at Monroe on January 7, 1928. Although green at the top at this time, the bases of the shoots were somewhat soft to the touch, and when sectioned were found to be entirely killed. The parenchymatous tissue was disorganized as shown in Figure 10. The cortex and epidermis were broken and the bacteria, held firmly between the cells when the plant was alive, were now gathered into masses, some of which had already passed out through rifts in the cortex and remained adherent to the exterior. A large part of the bacteria were in a position to be washed out of the collapsed tissue by rain and carried away in the surface water. The bacteria in the frost-shattered stems grew abundantly in culture.

The extent to which the parasitic bacteria are released from diseased plants by frost action and the importance of the bacteria released by this method in the spread of the disease must be determined by future work. Unfortunately, the development of the bacteria in these stem bases and their release is not easily observed without cytological examination. However, the conditions observed at Monroe and described in the preceding paragraph do not appear to be unusual. A similar though less extensive development of bacteria was found in the bases of stems of Grimm and other varieties of alfalfa at Madison, Wis., where the autumn shoots did not appear to develop as far as at Monroe. A similar infestation of autumn stems or stolons of Semipalatinsk alfalfa seems to have taken place at Madison in the preceding autumn. The plants involved in this instance were transplanted roots sent to the writer by N. E. Hansen in the spring of 1926 for the purpose of determining the resistance of this variety to wilt. The roots were set in a field near diseased plants. In the autumn young shoots showed evidence of disease, but the plants were still alive in the spring. A sloughing of the outer bark of the underground stems or stolons indicated winter injury.

The vessels and parenchymatous tissue of all the stems sectioned were found to be invaded by the parasitic bacteria as shown in Figure 5. The position of the bacteria in the innermost vessels indicated that they had entered the shoots in the autumn, and the bacteria in the outermost parenchymatous tissue were clearly being released into the soil following the decay of the tissue killed in the winter. The sloughing of the primary cortex, which is common in the spring,

FIG. 10.—Photomicrograph of a portion of a cross section of a crown stem of a 4-year-old Grimm alfalfa plant infected with bacterial wilt, collected January 7, 1928. The plant was taken from the same field from which the crowns shown in Figure 8 were taken. The disorganized condition of the parenchymatous tissue is the result of frost action. The stem is probably entirely killed at this time. The separation of the parenchymatous cells characteristic of freezing injury allows the bacteria formerly held in small masses between the cells to aggregate in larger ones, and the breaking of the outer tissue permits them to be washed out into the surface water. × 100

serves to release the bacteria over a longer period of time, and perhaps quite as effectually as the killing of the entire shoot. Thus, in view of what has been learned of the action of frost on the tissue of the alfalfa plant and the evidence cited here that the bacteria are often in a position to be freed from the tissue of diseased plants by this frost action, it appears to the writer that this method of escape is an important stage in the cycle of parasitism of this organism.

CYCLE OF PARASITISM OF APLANOBACTER INSIDIOSUM IN RELATION TO THE ALFALFA PLANT

From the evidence assembled in this paper, the life cycle of the bacteria producing wilt appears to be as follows: First, the bacteria develop abundantly in the autumn in all invaded parts of the diseased plants, but especially in the bases of young shoots that are injured or killed by freezing during the winter. Second, the bacteria are released from the stems by the action of frost in separating the cells of the parenchymatous tissue in which the bacteria are held and in breaking the cortex. Third, the bacteria are then distributed in surface water to other alfalfa plants. Fourth, in the spring the bacteria enter cracks in parenchymatous tissue opened by frost or by growth subsequent to frost injury and produce infection. The infected plants produce diseased stems in the following or second following autumn and from them frost action again releases the bacteria in the repetition of the cycle.

The observed behavior of the disease appears to be entirely in accord with this cycle of parasitism. Evidence that spring infection is the rule has already been given. The rapid spread of the disease in infested fields that have suffered severe winter injury has been observed repeatedly. The spread of the disease along the course taken by surface water has also been observed.

It is obvious, however, that other modes of dissemination and invasion contribute to the spread of the disease. Their relative importance remains to be determined and probably varies both locally and from year to year. Distribution of the bacteria by the knife of the mower is undoubtedly not uncommon. Chewing insects are under suspicion as conveyors of the bacteria and as the cause of wounds through which they may enter plants.

Sweet clover is known as another though probably unimportant host for the organism, and still other host plants may yet be found. It may be noted here that the behavior of the bacteria in a few sweet-clover plants has been studied and appears to be substantially the same as in alfalfa. The parenchymatous tissue of fall and early-spring growth of sweet clover is perhaps even more readily invaded than that of alfalfa, while its summer wood is more resistant.

DISCUSSION OF RESULTS

In the study of the development of the bacteria in the tissue of diseased plants as seen in cross sections of roots, the fact that bacteria develop more abundantly both in the parenchymatous tissue and in the vessels of wood produced in the autumn has been interpreted as indicating a high degree of susceptibility of autumn wood to bacterial invasion in comparison with summer wood. The precise character of this susceptibility is not obvious. It seems to be associated with the comparative absence of fibers. It is approximately coextensive with the region that is most susceptible to winter injury in the vascular bundles. It may be due to physical or chemical characteristics of the cell walls. Whatever its nature, it is obvious that if the alfalfa plant did not produce so much autumn wood in the root and crown it might be highly resistant to the disease.

If the sections of roots of the plants from Abilene, Kans., which recovered from the disease, are now examined with this in mind, it

is seen at once that the annual rings are very narrow indeed and have very little characteristic autumn wood, so little that the annual growth can not always be made out. The recovery from or endurance of the disease in these plants may have been due to resistance produced by environmental conditions affecting the character of growth, the most conspicuous effect being reduction in the amount of autumn wood. Furthermore, a few collections of roots from dry localities where only one or two crops of hay are cut each year show in most instances very little distinguishable autumn wood; and thus the observed fact that the disease never occurs in such fields except in low moist spots may be due not only to absence of water whereby the bacteria may be distributed, but also to actual resistance of the plants when infected. In further study of the disease the effect of environmental conditions upon the character of growth produced and the susceptibility of that growth to bacterial invasion should be tested.

If the most important cycle of parasitism of the parasitic bacteria proves upon continued investigation to be dependent upon winter injury to furnish wounds through which infection may take place in the manner described here, and if the bacteria do not live long in the soil or in other hosts a satisfactory control of the disease can undoubtedly be achieved by preventing the washing of the bacteria from old diseased fields to new seedings. This may involve the destruction of old stands in which the disease is abundant, when these are so located that surface water passing across them floods other fields. Old alfalfa fields when plowed up should not be reseeded until all plants that may carry the disease have been destroyed. Even when these precautions are taken, it appears that the disease may enter new fields located in infested localities, but not as early or as abundantly as when water can convey the bacteria readily from diseased plants.

In the absence of resistance in present varieties, and with uncertain success by the use of disease-escaping hardy varieties, a critical examination of the epidemiology of the disease is needed to define more precisely the cultural practices that may eliminate or reduce the disease in the several widely different agricultural districts where it has become an important enemy of the alfalfa crop.

SUMMARY

In the course of the study of bacterial wilt of alfalfa, observation of the disease in the field has suggested that winter injury of alfalfa plants provides wounds through which the bacteria often enter the plant. Following this suggestion, the relation of the bacteria to the host plant has been examined, and in this paper this relation and the effect of freezing injury upon the development of the disease are described.

The yellow discoloration of the wood of the root characteristic of the disease is due partly to a yellow insoluble material in the vessels occupied by or in the vicinity of the bacteria and partly to a relatively soluble stain that may diffuse for some distance from the infected region. Therefore the bacteria are usually not so widely distributed as the stain. In stems the stain does not develop so abundantly as in roots.

The gum formed in vessels near the bacteria appears to be a product of the plant, not of the bacteria.

The bacteria pass more readily from wounds in the phloem between the cells of the phloem rays, ray cambium, wood rays, and wood parenchyma into vessels.

In the vessels the bacteria are carried by water long distances vertically through open passages, and some open communication in a tangential direction permits to some degree distribution around the circumference of the root. The bacteria seem to advance through successive layers of wood by invading young parenchymatous tissues of the crown and the upper part of the root.

Parenchymatous cells do not permit intracellular invasion until they are nearly fully expanded though they are sometimes broken down with the formation of bacterial pockets. Parenchymatous cells of autumn and perhaps of early-spring growth admit of extensive intercellular invasion in autumn and spring but appear to become resistant in summer. Summer tissue, especially of the wood, appears to be relatively resistant to invasion at all times.

Seed infection has not been demonstrated, although the bacteria may be found far up the stems of seed-bearing plants and in one plant were identified in the base of a seed pod.

The symptoms of disease in the foliage of plants are associated with a considerable plugging of the vessels in the zone of wood that is active in conducting water, and in very young plants death sometimes appears to follow from such plugging. In plants of several years of age in the field a considerable invasion of parenchymatous tissue about the fascicular cambium of some of the bundles and a cessation of growth from the cambium usually precede death.

From cytological examination of roots of diseased plants collected in many fields it appears that at least 75 per cent of the plants examined thus far were infected in the spring. Although the longevity of infected plants varies greatly, it appears that the greater number show conspicuous symptoms of disease and die in the second year after infection.

Evidence is presented indicating that the prevalence of spring infection referred to previously is due to the entrance of the bacteria through wounds caused by winter injury.

An important source of the bacteria causing spring infection may be found in old diseased plants where the organism has developed abundantly in the bases of shoots produced in the autumn. Freezing injury or winterkilling of these shoots releases the bacteria into the surface water that may carry them to other plants.

If the cycle of parasitism outlined here is the most important method whereby the bacteria are distributed in epidemics of the disease, it appears that bacterial wilt may be greatly delayed or avoided by placing new seedings where they are inaccessible to surface wash or flooding by water that has passed among diseased plants.